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The Bethesda-Ballerup Group Of Paracolon Bacteria

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Introduction

Considerable attention has been drawn to the Bethesda and the Ballerup groups of paracolon bacteria since, upon preliminary biochemical examination, they resemble members of the genus *Salmonella*. Interest in the groups has been heightened further by the tendency of some workers to attribute etiological significance to these bacteria in enteric infections. Both groups can be classified in the *Paracolobactrum intermedium* group of Borman, Stuart, and Wheeler (*1*) and resemble the more strictly defined type 14011 of Stuart and his co-workers (*2*).

The present work was undertaken primarily for two reasons: first, to obtain sufficient information to define the two groups and to prepare satisfactory polyvalent serums for screening; and, second, to establish adequate methods for the recognition of serologic types so that it would be possible to study the occurrence and incidence of such types and thus gain some insight into their relation to enteric infections.

Early in the work it became apparent that no constant differences existed in the biochemical characteristics of the two groups. Furthermore, as the cultures were studied more closely, it was found that the type strains of some Bethesda O groups were related to type strains of described Ballerup O groups. Flagellar relationships between the type strains of the two groups also were found. As additional strains having similar biochemical properties were studied, the two groups were found to merge further serologically, since cultures with flagellar antigens typical of Ballerup strains were found within previously established Bethesda O groups. As new somatic groups were recognized, some strains of these O groups possessed flagellar antigens of the Bethesda group, while others displayed flagellar antigens of the Ballerup group.

In light of the above-mentioned observations it became obvious that the bacteria actually formed one large group of paracolon bacteria with similar biochemical properties and interlocking antigens. Therefore, they were combined into one group, herein referred to as the Bethesda-Ballerup group, and an intensive study of their antigenic relationships was begun. The study resulted in the establishment of an antigenic schema in which are included 32 O groups, 75 distinct combinations of H antigens, and 167 serologic types. In all, 506 cultures were employed in the study. Among cultures of this group encountered in diagnostic work, the O antigens of 95 percent and the H antigens of 85 percent corresponded to those established in this work.

The bacteria were found in man, in the lower mammals, and in fowl. Although it was not possible to make positive statements regarding their role in the production of enteric disease, it was noted that they occurred frequently in asymptomatic individuals.

History

The first-described strain of the Ballerup group was the organism called *Salmonella ballerup* by Kauffmann and Moeller (*3*). The organism behaved biochemically as a typical *Salmonella* culture and contained the Vi antigen of *Salmonella typhi* (*4*) and of *Salmonella paratyphi C* (*5*). Subsequently, other strains were studied, some of which were serologically identical with, and others closely related to, the original Ballerup culture, but they deviated widely in their biochemical properties from the typical pattern displayed by members of the genus *Salmonella*. *S. ballerup* therefore was removed from the genus *Salmonella* and formed the nucleus of a new group of enteric bacteria.

Harhoff (*6*) selected for intensive biochemical and serologic study 24 cultures which possessed O antigens related to those of the Ballerup type and established three subgroups on the basis of their constituent O antigens. Eight of these 24 strains fermented lactose, 1 fermented sucrose, and 15 utilized salicin within 6 to 14 days. No intensive investigation of the flagellar antigens was made by Harhoff, although cross-reactions between some strains were noted in reciprocal H agglutination tests. Bruner, Edwards, and Hopson (*7*) extended the work of Harhoff in a study of 45 strains and recognized 2 additional somatic groups. Twenty-one serotypes were established on the basis of O and H antigens. The majority of the 45 strains utilized lactose after prolonged incubation in sealed tubes or after serial transfer in lactose broth over a period of several months. Twenty-two of the strains fermented sucrose. Nine strains failed to ferment either lactose or sucrose, whereas all except 2 strains fermented salicin within 1 to 19 days.

The biochemical and serologic study of the Bethesda group as such was reported first by Edwards, West, and Bruner (*8*). In the hope of establishing the etiological significance of groups of epidemiologically related strains of these bacteria, these investigators made an intensive antigenic study of 32 strains from 4 outbreaks of diarrhea. Of these 32 cultures, 22 were isolated from 12 patients in 1 outbreak, 4 from 4 patients in another episode, and 2 from 2 patients in a third outbreak. The remaining 2 cultures were isolated from milk and from a person who drank it. These strains formed a fairly uniform biochemical pattern and were divisible into 4 somatic groups representing 8 serologic types. The majority of strains fermented lactose within 3 to 6 days. Sucrose was utilized by 11 of these Bethesda strains while salicin was fermented by about one-half of them after 25 days' incubation. Dulcitol was utilized by all of the strains.

No definite conclusions as to the role of these cultures in an infectious process could be reached by the data obtained in that study, although there was some agreement between the epidemiological data and the types established by serologic examinations. One serologic type was present in 75 percent of the individuals involved in 1 outbreak, and in a second outbreak 3 of 4 persons affected yielded identical cultures. Asymptomatic food handlers involved in the latter outbreak, however, yielded distinctly different types. Examination of cultures from individuals with no history of diarrhea showed that the incidence of Bethesda paracolons in asymptomatic individuals was quite high. Moran and Bruner (*9*) continued the study of members of the Bethesda group and demonstrated 9 O groups and 25 serologic types. Their observations on the cultural characteristics were essentially the same as those established by Edwards and his co-workers (*8*). However, seven of their strains failed to ferment dulcitol.

Investigation of the salmonella-like paracolons, and the Bethesda group in particular,

was continued by the authors when it became evident from the examination of cultures submitted to the laboratory for diagnosis that existing serologic classifications did not provide adequate antigenic coverage of this biochemical group of enteric bacteria. The lack of adequate methods for the rapid screening and the exact typing of these micro-organisms led to a considerable lapse of time before many of them could be eliminated from the *Salmonella* group and made it impossible to study their potential role in enteric infections.

Materials and Methods

Cultures Studied

The test strains used in this study, 35 O and 75 H type strains, together with their sources and histories when known, are listed in tables 1 and 2, respectively. An additional 396 cultures were included in the study. The sources of the latter cultures are not listed individually but are indicated according to serologic type in table 29, in which the antigenic schema is presented. In many instances the sources and histories of the cultures were not known. The majority of the strains of O group 29 were received from Dr. D. W. Bruner and were those described by him and his associates (*7*).

Biochemical Tests

All 506 cultures were tested for the production of hydrogen sulfide in 2 percent peptone water with lead acetate papers as indicator, for indol formation by Kovac's method, for urease production in Christensen's urea agar, and for the utilization of Simmons' citrate and Jordan's tartrate. The Voges-Proskauer and methyl red reactions were determined in Difco MR–VP medium after incubation for 2 and 4 days, respectively. O'Meara's method was used for the detection of acetyl-methyl-carbinol. The utilization of glucose, sucrose, lactose, dulcitol, and salicin was determined in nutrient broth containing these substrates with Andrade's reagent as an indicator. All fermentation tubes which were negative after 48 hours' incubation were sealed with sterile paraffined corks and reincubated for 30 days before being discarded as negative.

The cultures on Christensen's urea agar and Simmons' citrate were held for 4 days before being discarded as negative.

In addition to the above tests, 200 of the cultures, including the 110 test strains, were tested for utilization of xylose, maltose, mannitol, arabinose, trehalose, rhamnose, and adonitol. The production of hydrogen sulfide (H_2S) in triple sugar iron agar (TSI) and also in the motility sulfide medium of Hajna (*10*) was determined, as were the reactions obtained in litmus milk and nutrient gelatin. These cultures were tested also for the reduction of nitrates.

The tests for hydrogen sulfide production and for the utilization of tartrate were read after overnight incubation. Nutrient gelatin stabs were incubated at 25° C. and observed over a period of 30 days. The reactions in litmus milk were observed over the same period of time.

Serum Production

The techniques employed in the preparation of antiserums were essentially the same as those reported in previous studies (*11*). With one exception, the O antiserums were prepared by the injection of 18-hour infusion broth cultures, which were heated at 100° C. for 2.5 hours and preserved with formalin. One culture (3723–51) contained an alpha-like antigen which was not completely inactivated after heating at 100° C. It was necessary to heat this culture at 121° C. for 2.5 hours in order to obtain a suitable O antiserum.

Flagellar antiserums were prepared by in-

jecting rabbits with formalinized 18-hour infusion broth cultures of actively motile organisms.

Antiserums for the Vi antigen of Felix and Pitt (*4*) were produced from the V forms of Coli 1 of Kauffmann (*5*), the Ballerup 107 strain of Kauffmann and Moeller (*3*), and the Watson strain of *S. typhi*. Plates seeded with V forms were incubated overnight at 37° C.; the growth was scraped off and suspended in absolute alcohol. The suspensions were centrifuged, the alcohol decanted, and the organisms dried in vacuo. The cells were ground to a fine powder, portions of which were suspended in physiological saline immediately before they were injected.

Antiserums for two additional heat labile antigens which masked O agglutination were prepared by 2 injections of increasing amounts of formalinized cultures, followed by 4 injections of living organisms. Fresh 4-hour broth cultures were prepared for each injection.

Agglutination and Agglutinin Absorption

O Antigens

In the study of O antigens, reciprocal agglutination tests were performed with all O type strains and O antiserums by the slide test, using alcohol-treated antigens prepared by the method of White (*12*). Throughout this study, such antigens were found to be eminently suitable for the determination of O groups as well as O types in slide agglutination tests, using appropriately diluted or absorbed antiserums. Reciprocal tube agglutination tests in

Table 1. O type strains and sources

O group	O antigens	Type strains	Source
1	1a,1b,1c	Na1A	Stool, adult, diarrhea outbreak.
2	2a,1b	Na4	Stool, adult, gastroenteritis outbreak.
3	3a,3b,1c	Na11	Stool, adult, gastroenteritis outbreak.
4	4a,4b	St1201	Stool, adult, gastroenteritis outbreak.
5	5a,5b,4b	3796	Stool, adult, asymptomatic; stock culture from Dr. D. W. Bruner.
6	6,4b,5b	StU260B	Stock culture from Dr. C. A. Stuart.
7	7,3b,1c	Md1	Stool, food handler.
8	8a,1c	Md3	Stool, food handler.
	8a,8b	Mich10	Stool.
	8a,8c	Mich11	Stool.
9	9a,9b	FtMac77	Stool, infant, diarrhea.
10	10,9b	2675/50	No history.
11	11	Ind1401	Stool.
12	12a,12b	Fla46	Stool, food handler.
	12a,12c	St18597	Stock culture from Dr. C. A. Stuart.
13	13	Ariz6379	Stool.
14	14	Gal660T	
15	15	Fla6	Stool.
16	16	Ariz2121	
17	17	Mich8	Stool.
18	18	NJ191	Stool.
19	19	Fla1589	
20	20	Ind101/50	Stool.
21	21a,21b	Ha616	Feces, rat.
22	22	Ind1219	Stool, adult, gastroenteritis.
23	23	Fla1532	
24	24	GaHill	Stool, food handler.
25	25	Na21	Stool, adult, gastroenteritis.
26	26	Tex 5819	Stool, adult.
27	27	3723/51	Stool, adult.
28	28,1c	Kauf18	Stool.
29	29	Bal107	Stool, adult, gastroenteritis; stock culture from Dr. D. W. Bruner.
30	30,21b	5818	Stool, adult, stock culture from Dr. D. W. Bruner.
31	31	Braz109	Stool.
32	32	Bonn6134	Stool, asymptomatic.

which boiled broth cultures were employed as antigens also were used to determine the extent of cross reactions within and among all O groups, and to determine residual titers after reciprocal agglutinin absorption experiments.

Absorption tests were done with the type strains of the various O groups in every instance in which antigenic relationships were indicated by cross-reaction in slide agglutination tests or by agglutination in tube tests at a dilution of 1:200 or above. The absorptions were performed with packed cells centrifuged from saline suspensions which had been heated at 100° C. for 1 hour. The packed cells were mixed with an appropriate dilution of the antiserum and the mixture was incubated at 48° to 50° C. for 1 hour. In many instances, a second absorption of the antiserum was necessary before all agglutinins for the absorbing strain could be removed.

After the antigens characteristic of the type strains of the various O groups were determined and absorbed antiserums were prepared where necessary, other Bethesda-Ballerup-like cultures were tested by slide agglutination. From those giving positive reactions, a representative number of cultures of each O group were selected for tube agglutination and agglutinin absorption tests to determine whether their O antigens were identical with, or merely related to, those of the O type strains. O antiserums were prepared with those cultures whose O antigens were related to, but not identical with, those of the O type strains. If the slide agglutination tests were negative, a dense saline suspension of the organism was boiled for the destruction of any masking antigens which might be present. The heated suspension then was retested in the O antiserums.

In determining the O relationships existing between the Bethesda-Ballerup group and other groups of enteric bacteria, alcohol-treated antigens of the Bethesda-Ballerup O type strains were used in slide tests with antiserums representing the O types of both the Arizona group of paracolon bacteria (*6*) and of the *Salmonella* group. In addition, boiled broth antigens of Bethesda-Ballerup O type strains were spot checked at a dilution of 1:100 in tube tests with antiserums representative of all known *Salmonella* O groups. When significant reactions occurred, either in the slide or the tube tests, the extent of each relationship was determined in reciprocal tube agglutination tests using boiled broth cultures as antigens.

Alcohol-treated antigens of the Arizona and *Salmonella* O type strains were tested on the slide in the O antiserums of the Bethesda-Ballerup group. Boiled broth antigens of Arizona and *Salmonella* O type strains were used in the tube agglutination tests with Bethesda-Ballerup O antiserums only where reactions occurred in slide tests or in instances in which a relationship between the groups was indicated by the reaction of Bethesda-Ballerup cultures in a given Arizona or *Salmonella* antiserum. Reciprocal agglutinin absorptions were performed in those instances in which tube agglutination occurred at a dilution of 1:200 or above.

In order to determine the O relationships which existed between the Bethesda-Ballerup O types and the presently recognized 124 *Escherichia coli* O groups, boiled broth antigens of the Bethesda-Ballerup strains were tested in tube tests with *E. coli* O antiserums. In those instances in which agglutination occurred at a dilution of 1:200 or above in the *E. coli* antiserums, reciprocal tube agglutinations were performed with the respective *E. coli* O antigens and Bethesda-Ballerup O antiserums. It is recognized that all *E. coli* O type strains must be tested in all the Bethesda-Ballerup O antiserums before a complete report can be made of the relationships existing between these two groups.

K Antigens

The presence of Vi antigen in Bethesda-Ballerup strains was determined in slide tests with living organisms using unabsorbed Vi antiserums prepared against *S. typhi* and against Coli 1. These reactions were controlled by the use of the appropriate Bethesda-Ballerup O antiserum as well as O antiserums prepared against *S. typhi* and Coli 1.

Antiserums for two additional masking antigens were freed of O and H agglutinins by absorption. The resulting absorbed K antiserums were titrated with a living 6-hour broth culture of the homologous organisms and read after incubation for 1 hour at 37° C. These absorbed antiserums were used subsequently in slide tests with living organisms

Table 2. H type strains and sources

H antigens	Type strains	Source
1,2	Na1A	Stool, adult, diarrhea, outbreak.
2,3	Fla 1995	No history.
4,5	Na2C	Stool, adult, diarrhea, outbreak.
5,6	Na23	Stool, adult, diarrhea, outbreak.
7,(8),10	Na4	Stool, adult, gastroenteritis outbreak.
8	Na22	Stool, adult, gastroenteritis outbreak.
8,9	Na11	Stool, adult, gastroenteritis outbreak.
8,10,11	Bal 14671	Stool, adult, gastroenteritis.
8,12	Fla1608	
(9),13,14	Md2	Stool, adult, diarrhea.
(9),13,15	Md3	Stool, food handler.
14,15,16	Ala20	Stool.
(13),17	Mich7	Stool.
(13),18,19	4216–50	Stool, nurse, diarrhea.
19,20	Md6	Stool, food handler.
21,22	Md10	Stool, typhoid carrier.
21,23	Ankers	
21,24	Mich8	Stool.
(21),25,26	Mich10	Stool.
(21),25,27	Ga267	Stool, food handler.
(23), 28	Bonn6134	Stool, adult, asymptomatic.
(9),29,30	Na921	Stool, adult, gastroenteritis.
(9),29,31	Mich1	Stool.
32,33	Mich11	Stool.
32,34	Conn2780	Stool.
35,36	Bonn16824	Stool, adult, gastroenteritis.
35,37	Ind6A	Blood, adult dying of amyloid infiltration of liver, spleen.
35,(36),38	Na21	Stool, adult, gastroenteritis.
39	Mich5	Stool.
40,41	Gal660T	
40,42	BalK59	Stock culture from Dr. D. W. Bruner.
40,41,43	Ind101–50	Stool.
44,45	St6125	Stock culture from Dr. C. A. Stuart.
44,46	St8380	Stock culture from Dr. C. A. Stuart.
47	Ga97	Stool, food handler.
48	Bonn4892	Stool, food handler.
49, 50	Tex5819	Stool, adult.
49, 51	Ga Hill	Stool, food handler.
52	Fla1532	
53, 54	BG4673–50	Stool, diarrhea.
53, 55	Bonn5937	Stool, adult, asymptomatic.
56	NJ191	Stool.
57	St18597	Stock culture from Dr. C. A. Stuart.
58	Ariz2121	Stool.
59	Ariz6379	Stool.
60	Ha616	Feces, rat.

Table 2. H type strains and sources—Continued

H antigens	Type strains	Source
61	Tex6406	Stool.
62	Fla6513	Turkey.
63	Balt6331–50	Stool, adult.
64	Ind1219	Stool, diarrhea.
65	GaStokes	Stool.
66	IdElder	Stool.
67	VaSingleton	Stool.
68	GaHolland	Stool.
69	4312	Stool, adult, diarrhea.
70	Kauff18	
71	Braz109	Stool.
72	StU260B	Stock culture from Dr. C. A. Stuart.
73	Bal107	Stool, adult, gastroenteritis.
74	Bal M	Stool.
75	Horm	Ovary, hen, stock culture from Dr. D. W. Bruner.
74, 75	LC54	Sewage, stock culture from Dr. D. W. Bruner.
76	5818	Stool, adult.
77	K527	Stock culture from Dr. D. W. Bruner.
77, 78	Ind1401	Stool.
77, 79	K8665	Stock culture from Dr. D. W. Bruner.
77, 80	H10	Stock culture from Dr. D. W. Bruner.
77, 81	20964	Stock culture from Dr. D. W. Bruner.
77, 88	G36	Stool, infant, diarrhea.
82	16385	Stool, adult, stock culture from Dr. D. W. Bruner.
83	222C–1	Stool, adult, gastroenteritis, stock culture from Dr. D. W. Bruner.
84	H–8	Stool, stock culture from Dr. D. W. Bruner.
85	17776	Stool, adult, salmonellosis, stock culture from Dr. D. W. Bruner.
86	2058	Stock culture from Dr. D. W. Bruner.
87	48203	Stool, gastroenteritis, stock culture from Dr. D. W. Bruner.

when testing for these O-inhibiting antigens in other Bethesda-Ballerup strains.

H Antigens

Each of the 75 H type strains was titrated in serial dilutions in tube tests with each of the 75 H antiserums. These tests were read after incubation for 1 hour at 48° to 50° C. If agglutination occurred in a dilution of 1:200 or above in heterologous serums, reciprocal absorption tests were done. In the flagellar agglutinin absorption experiments, O agglutinins were removed from all H antiserums by absorbing the serum with a heated suspension of the homologous strain. Packed cells from heated saline suspensions were mixed with dense formalinized saline suspensions of the absorbing flagellar strains and added to appropriately diluted H antiserums for absorption. In all instances 2 serial absorptions were carried out

at 48° to 50° C. for 1 hour with an excess of the flagellated absorbing organism.

After the relationships among the H type strains were determined and suitable absorbed antiserums were prepared, additional Bethesda-like strains were tested in the H antiserums. One of the difficulties encountered in the antigenic analysis of Bethesda-Ballerup strains was the inability to obtain floccular H type agglutination with many of the strains, due to their poor motility and the poor development of H antigens. This necessitated repeated passage of such strains through semisolid agar before an analysis of their flagellar antigens could be made. After maximum motility of the cultures was assured, 18-hour infusion broth cultures were formalinized for H antigens. These were tested first in pooled H antiserums so arranged that each of the H type serums was present in a final dilution of 1:1,000.

If agglutination occurred in the pooled antiserums, the antigens then were tested in the indicated individual H antiserums at a dilution of 1:1,000. The exact H type was then determined by the use of appropriate absorbed H antiserums. In each instance in which identification of the flagellar antigens resulted in a new serologic type, i. e., a combination of O and H antigens not previously recognized, titrations and agglutinin absorptions were performed with the indicated antiserums. In a few instances flagellar antiserums were prepared against heterologous strains even though these strains, by agglutinin absorption tests, removed all H agglutinins from a particular H type antiserum. This was done in order to determine whether additional H antigens were present in the heterologous strains.

In order to determine the relationship of the flagellar antigens of the Bethesda-Ballerup group to those of other enteric bacteria, the 75 flagellar antigens of the Bethesda-Ballerup group were tested at dilutions of 1:200 and 1:500 in antiserums representing all of the recognized H antigens of monophasic and diphasic strains of the Arizona group, the *Salmonella* group and the *E. coli* group. They also were tested in antiserums prepared against six induced phases of *Salmonella* organisms, including three induced phases of *S. paratyphi A*, and one induced phase each of *S. typhi*, *Salmonella minnesota*, and *Salmonella abortus equi*. Complete reciprocal titrations were carried out when indicated.

Results

Biochemical Tests

All of the cultures blackened lead acetate papers, were indol negative, methyl red positive, Voges-Proskauer negative, failed to hydrolyze urea, acidified Jordan's tartrate agar, and were positive on Simmons' citrate agar within 1 to 3 days.

All fermented glucose promptly, usually with the production of gas. Forty-five strains utilized sucrose; 244, lactose; 188, dulcitol; and 104, salicin. Thirty-six strains which were negative in lactose and sucrose were positive in salicin, in most cases within 1 day. Sixteen strains failed to utilize sucrose, lactose, or salicin within a 30-day period.

Of the 200 cultures tested, none liquefied gelatin nor fermented adonitol. All reduced nitrates to nitrites and promptly fermented arabinose, xylose, maltose, trehalose, rhamnose, and mannitol. The reactions in litmus milk seemed to parallel the fermentation of lactose. In a few instances among the rapid lactose fermenters, coagulation of litmus milk occurred.

The comparison of hydrogen sulfide production by various methods has some practical applications in routine diagnosis. With very few exceptions, strong reactions were obtained in 2-percent peptone water with lead acetate paper as the indicator. These results were paralleled by reactions obtained in Hajna's motility sulfide medium. In a number of instances, however, where strongly positive reactions were obtained by these two methods, negative or only faintly positive hydrogen sulfide reactions were obtained in the TSI agar.

Failure to blacken TSI agar does not exclude a culture from the Bethesda-Ballerup group.

Serologic Reactions

O Antigens

O antigens of the Bethesda-Ballerup group. By agglutination and agglutinin absorption tests, 32 O groups and 35 O types were recognized. In general, the somatic antigens were specific, and few intergroup reactions were observed. The exceptions to this were found largely within O groups 1 to 10, inclusive. Since a table which included the results of all reciprocal O tube agglutination tests would be extremely cumbersome, these reactions are not delineated. Instead, the cross agglutinations occurring among O groups and O types are set forth in a series of small tables in which also are included the results of the agglutinin absorption tests which were necessary to establish the relationships among the O antigens. Minor relationships occurring between and among somatic groups were represented by antigenic symbols only when their presence was evident in slide agglutination tests and caused confusion in O group determination. The results of slide agglutination tests are shown in table 3. For the O group type strains and their formulas reference is made again to table 1.

Table 4 presents the relationships which exist between O groups 1, 2, 3, 7, 8, and 28 and shows the absorptions necessary for the preparation of group specific antiserums.

O groups 1 and 2 have strong reciprocal relationships. The strong reactions between these two groups made necessary the use of absorbed antiserums to establish their identity. O group 1 antiserum containing the agglutinins 1a, 1b, 1c was absorbed by the O group 2 type strain (2a,1b) for the preparation of the diagnostic serum for O group 1.

The diagnostic serum for O group 2 was obtained by the absorption of that antiserum by the O group 1 type strain. The O group 1 type strain showed one-sided relationships with O groups 3 and 7 and with one subtype of group 8 (8a,1c). The fraction indicating these relationships was designated as 1c. The type strains of groups 3 (3a,3b,1c), 7 (7,3b,1c), and one subtype of group 8 (8a,1c) did not agglutinate in the antiserum of O group 1 at a dilution of 1:100. Groups 3 and 7 shared an additional factor evidenced by reciprocal agglutination reactions. This relationship was represented by the symbol 3b. In order to obtain an antiserum specific for group 3 strains it was necessary to absorb group 3 antiserum both by group 1 and group 7 type strains, since the 1c fraction of group 7 strains was not present in sufficient quantity to remove all 1c agglutinins from O group 3 antiserum.

Serum specific for group 7 strains was obtained by the absorption of group 7 antiserum with the group 3 type strain which apparently contained relatively large portions of the nonspecific fractions shared with group 7.

Group 8 represents three different subtypes with complicated antigenic structures. Not all of the antigenic fractions present are expressed in the symbols assigned to members of this O group. It has been the aim in presenting the results of this study to adhere to as simplified a system of designation of antigenic fractions as was practical for type determination. As can be seen in table 4, a group-specific serum agglutinating all subtypes (8a,1c; 8a,8b; 8a,8c) within O group 8 was prepared by the absorption of 8a,1c antiserum with the type strain of O group 1. For the determination of subtype within O group 8, other absorbed antiserums were necessary. Single factor 8b was prepared by the absorption of 8a,8b antiserum by the 8a,8c type strain, whereas single factor 8c was prepared by the absorption of 8a,8c antiserum by either 8a,1c or 8a,8b type strains. As is evident from the data, there is an unexpressed minor relationship between the type strains 8a,8b and 8a,8c which is not shared by the 8a,1c type strain. When 8a,8b antiserum was absorbed by 8a,1c type strain, agglutinins for 8a,8c type strain were still demonstrable. In addition, although both 8a,1c and 8a,8b type strains may be used for the absorption of 8a,8c antiserum in the preparation of the specific fraction 8c, the 8a,8b strain removed a significantly greater proportion of the specific agglutinins than did the 8a,1c strain.

A small unexpressed specific fraction for O type 8a,1c remained for the homologous strain after the absorption of its antiserum by a group 1 strain plus either of the two other subtypes within O group 8. The described fraction can be

Table 3. Slide agglutination tests among Bethesda-Ballerup O groups and O types

Antigens	O antiserums																	
	1a,1b,1c	2a,1b	3a,3b,1c	4a,4b	5a,5b,4b	6,4b,5b	7,3b,1c	8a,1c	8a,8b	8a,8c	9a,9b	10,9b	11	12a,12b	12a,12c	13	14	15
1a,1b,1c	++	±	+	−	−	−	++	+	−	−	−	−	−	−	−	−	−	−
2a,1b	+	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3a,3b,1c	−	−	++	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−
4a,4b	−	−	−	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5a,5b,4b	−	−	−	−	++	+	−	−	−	−	−	−	−	−	−	−	−	−
6,4b,5b	−	−	−	±	+	++	−	−	−	−	−	−	−	−	−	−	−	−
7,3b,1c	−	−	±	−	−	−	++	−	−	−	−	−	−	−	−	−	−	−
8a,1c	−	−	−	−	−	−	−	++	++	+	−	−	−	−	−	−	−	−
8a,8b	−	−	−	−	−	−	−	±	++	+	−	−	−	−	−	−	−	−
8a,8c	−	−	−	−	−	−	−	±	+	++	−	−	−	−	−	−	−	−
9a,9b	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−	−	−
10,9b	−	−	−	−	−	−	−	−	−	−	±	++	−	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−
12a,12b	−	−	−	−	−	−	−	−	−	−	−	−	−	++	++	−	−	−
12a,12c	−	−	−	−	−	−	−	−	−	−	−	−	−	++	++	−	−	−
13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−
14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++
16	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
19	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
21a,21b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
22	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
23	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
26	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
27	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
28,1c	−	−	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
29	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
30,21b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
31	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

demonstrated in slide agglutination tests as well as in tube agglutination tests.

The O type strain 28,1c showed a very strong one-sided relationship to the type strain of O group 3. It was agglutinated by antiserum 3a,3b,1c to 50 percent of the homologous titer of that serum, yet the O group 3 type strain was not agglutinated at a dilution of 1:100 by 28,1c antiserum. The 28,1c type strain was agglutinated to 10 percent of the titer of 7,3b,1c antiserum, yet the 7,3b,1c type strain was not agglutinated by 28,1c antiserum. The O type strain 1a,1b,1c was agglutinated to 10 percent of the titer of 28,1c antiserum, although the reverse reaction did not occur. The type strains of O groups 2, 3, 7, and 8 were not agglutinated at a dilution of 1:100 by the antiserum for O group 28. When 28,1c antiserum was absorbed by type strain 1a,1b,1c a fraction specific for the O group 28 type strain remained.

The relationships which existed between O groups 4, 5, and 6 are presented in table 5. The specific fraction for O group 4 could be prepared by the absorption of group 4 antiserum by strains of either O group 5 or O group 6. The specific fraction for O group 5 was prepared by the absorption of group 5 antiserum by a group 6 antigen, while the specific fraction for O group 6 was obtained by absorbing group 6 antiserum by a group 5 antigen.

O groups 9 and 10 shared a common factor, 9b. As is evident in table 6, this relationship between the two groups was a minor one, yet it was apparent in slide agglutination tests. The specific factor serum for O group 9 was prepared by absorbing group 9 antiserum with a

Table 3. Slide agglutination tests among Bethesda-Ballerup O groups and O types—Continued

Antigens	O antiserums																
	16	17	18	19	20	21a,21b	22	23	24	25	26	27	28,1c	29	30,21b	31	32
1a,1b,1c	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
2a,1b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3a,3b,1c	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−
4a,4b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5a,5b,4b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6,4b,5b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7,3b,1c	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
8a,1c	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
8a,8b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
8a,8c	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
9a,9b	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−
10,9b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12a,12b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12a,12c	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	−	−	−	−	−	−	−	−	−	−	−	−	tr	−	−	−	−
16	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	−	−	++	−	−	−	−	−	−	−	−	−	++	−	−	−	−
19	−	−	−	++	−	−	−	−	−	−	−	−	−	−	−	−	−
20	−	−	−	−	++	−	−	−	−	−	−	−	−	−	−	−	−
21a,21b	−	−	−	−	−	++	−	−	−	−	−	−	−	−	+	−	−
22	−	−	−	−	−	−	++	−	−	−	−	−	−	−	−	−	−
23	−	−	−	−	−	−	−	++	−	−	−	−	−	−	−	−	−
24	−	−	−	−	−	−	−	−	++	−	−	−	−	−	−	−	−
25	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−	−	−
26	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−	−
27	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−
28,1c	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−	−
29	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−	−
30,21b	−	−	−	−	−	+	−	−	−	−	−	−	−	−	++	−	−
31	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−
32	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++

− No agglutination in diagnostic dilution of serum.
±, +, ++ Increasing degrees of agglutination.
tr—Trace.

strain of O group 10. A group 9 strain was used for absorption of group 10 antiserum in preparing the specific factor of that group.

Two subtypes were represented in O group 12. The degree of relationship between these two types within the group and the preparation of type specific antiserums are given in table 7. Type strain 12a,12b was agglutinated to the homologous titer of 12a,12c antiserum, whereas the 12a,12c type strain was agglutinated only to 10 percent of the titer of 12a,12b antiserum.

O groups 21a,21b and 30,21b were related reciprocally through a minor antigen 21b. The cross agglutination reactions and the results of the agglutinin absorption experiments between these two O group strains are presented in table 8. In the tube agglutination test, strain 21a,21b was agglutinated only to 1:200 in antiserum 30,21b; and strain 30,21b was agglutinated in antiserum 21a,21b only to 1:100. The homologous titer of neither antiserum was reduced by absorption with the heterologous strain. This minor relationship was expressed by symbols because of the strong cross reactions between these two strains and their respective antiserums in the slide tests.

Somatic group 11, groups 13 through 27 inclusive, and groups 29, 31, and 32 showed no significant cross-reactions with other groups, and identification of their antigens can be accomplished by the use of unabsorbed antiserums in appropriate dilutions.

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Table 4. Relationships among O groups 1, 2, 3, 7, 8, and 28

O antiserums	O antigens							
	1a,1b,1c	2a,1b	3a,3b,1c	7,3b,1c	8a,1c	8a,8b	8a,8c	28,1c
1a,1b,1c:								
Unabsorbed	5, 000	1, 000	----	----	----	----	----	----
Absorbed by 2a,1b	5, 000	----	----	----	----	----	----	----
2a,1b:								
Unabsorbed	500	5, 000	----	----	----	----	----	----
Absorbed by 1a,1b,1c	----	5, 000	----	----	----	----	----	----
3a,3b,1c:								
Unabsorbed	2, 000	----	10, 000	2, 000	----	----	----	5, 000
Absorbed by 1a,1b,1c + 7,3b,1c	----	----	5, 000	----	----	----	----	----
7,3b,1c:								
Unabsorbed	200	----	500	5, 000	100	----	----	500
Absorbed by 3a,3b,1c	----	----	----	2, 000	----	----	----	----
8a,1c:								
Unabsorbed	1, 000	----	----	----	5, 000	2, 000	2, 000	----
Absorbed by—								
1a,1b,1c	----	----	----	----	2, 000	2, 000	2, 000	----
1a,1b,1c+8a,8b	----	----	----	----	200	----	----	----
1a,1b,1c+8a,8c	----	----	----	----	200	----	----	----
8a,8b:								
Unabsorbed	100	----	----	----	1, 000	5, 000	5, 000	----
Absorbed by—								
8a,1c	----	----	----	----	----	2, 000	500	----
8a,8c	----	----	----	----	----	1, 000	----	----
8a,8c:								
Unabsorbed	----	100	----	----	500	1, 000	2, 000	----
Absorbed by—								
8a,1c	----	----	----	----	----	----	1, 000	----
8a,8b	----	----	----	----	----	----	200	----
28,1c:								
Unabsorbed	500	----	----	----	----	----	----	5, 000
Absorbed by 1a,1b,1c	----	----	----	----	----	----	----	500

NOTE: In this, and in subsequent tables giving results of agglutination tests, ---- indicate absence of agglutination at 1: 100.

O group 29 represented all of the serotypes of the original Ballerup O group 1 strains described by Bruner and associates (*7*). These workers, as Harhoff previously had done, were able to distinguish subtypes within a closely knit and complex O antigen group. These subtypes within O group 29 were not indicated in the present schema. An antiserum prepared against any one of the five subtypes of the group gave strong slide reactions with all types within the group. No cross reactions occurred between O group 29 and other O groups of Bethesda-Ballerup organisms and, since only one subtype within the group could be demonstrated in slide agglutination tests with our absorbed antiserums, it was felt that, for practical purposes, one symbol should be assigned to all members of this O group. The inability to demonstrate the specific fractions for all subtypes within this O group by slide tests was noted by Bruner, Edwards, and Hopson. Most of these fractions, according to these authors, were apparently too weak to be manifested except in tube agglutination tests. Reference is made to table 9, which includes the antigenic symbols and formulas assigned to Bethesda-Ballerup O groups in this and previous studies.

The absorptions necessary for the preparation of specific O grouping and typing serums are summarized in table 10. Only those O groups and O types involved in intergroup and intertype relations are included in the table. As

Table 5. Relationships among O groups 4, 5, and 6

O antiserums	O antigens		
	4a,4b	5a,5b,4b	6,4b,5b
4a,4b:			
Unabsorbed	2, 000	100	100
Absorbed by—			
5a,5b,4b	2, 000		
6,4b,5b	2, 000		
5a,5b,4b:			
Unabsorbed	100	2, 000	1, 000
Absorbed by—			
4a,4b		1, 000	1, 000
6,4b,5b		1, 000	
6,4b,5b:			
Unabsorbed		200	2, 000
Absorbed by 5a,5b,4b			1, 000

Table 6. Relationships between O groups 9 and 10

O antiserums	O antigens	
	9a,9b	10,9b
9a,9b:		
Unabsorbed	2, 000	200
Absorbed by 10,9b	2, 000	
10,9b:		
Unabsorbed	200	2, 000
Absorbed by 9a,9b		1, 000

mentioned above, the remaining O groups were determined with unabsorbed serums.

Relationships of O Antigens to Those of Other Enteric Bacteria. In general, the O relationships which existed between the Bethesda-Ballerup group organisms on the one hand and those of the Arizona, *Salmonella*, and *E. coli* groups on the other were minor ones, as evidenced by the extent of reactions in tube agglutination tests. However, the O reactions between the Bethesda-Ballerup and the Arizona and *Salmonella* groups were sufficiently strong in slide agglutination tests to cause confusion as to bacterial group identity in simplified screening tests. The reactions of Bethesda-Ballerup strains in slide tests with antiserums of the *Salmonella* and Arizona groups are given in table 11, and the cross agglutination of the *Salmonella* and Arizona O type strains in Bethesda-Ballerup O antiserums are presented in table 12. The *Salmonella* and Arizona O antiserums and antigens were placed together in these tables of cross agglutination reactions with Bethesda-Ballerup O antigens and antiserums. There are known strong relationships and cases of identity between some of the O antigens of the *Salmonella* and Arizona groups (*13*). Therefore, the data were consolidated to eliminate duplication and to emphasize the significance of parallel relationships which existed among these three groups of enteric bacteria. Since slide agglutination tests are used so widely, the results of such tests with the O antigens of Bethesda-Ballerup type strains in *Salmonella* and Arizona O antiserums were stressed even though the tube agglutination tests gave a clearer idea of the extent and degree of these intergroup relationships. In some instances, the relationships evident in slide tests were not reciprocal ones.

By referring to tables 11 and 12 the slide reactions between these three groups can be seen. Bethesda-Ballerup type strain 9a,9b was agglutinated strongly in *Salmonella* XXX antiserum and the reciprocal reaction also was

Table 7. Relationships within O group 12

O antiserums	O antigens	
	12a,12b	12a,12c
12a,12b:		
Unabsorbed	2, 000	2, 000
Absorbed by 12a,12c	200	
12a,12c:		
Unabsorbed	200	2, 000
Absorbed by 12a,12b		500

Table 8. Relationships between O groups 21 and 30

O antiserums	O antigens	
	21a,21b	30,21b
21a,21b:		
Unabsorbed	2, 000	100
Absorbed by 30,21b	2, 000	
30,21b:		
Unabsorbed	200	1, 000
Absorbed by 21a,21b		1, 000

Table 9. Antigenic formulas assigned Bethesda-Ballerup O type strains in previous reports

Suggested designation				
Bethesda-Ballerup West, Edwards 1954	Bethesda Moran, Bruner 1949	Bethesda Edwards, West, Bruner, 1948	Ballerup Harhoff 1949	Ballerup Bruner, Edwards, Hopson 1949
1a,1b,1c	1,2	1	—	—
2a,1b	1,3	2	—	—
3a,3b,1c	1,4,5	3	—	—
4a,4b	11	4	—	—
5a,5b,4b	—	—	—	2
6,4b,5b	—	—	—	—
7,3b,1c	5,6	—	—	—
8a,1c	2,7	—	—	—
8a,8b	7,8,9	—	—	—
8a,8c	7,8,10	—	—	—
17	12	—	—	—
29	—	—	$XXIX_1$, $XXIX_2$	1a,1b
29	—	—	$XXIX_1$, $XXIX_2$, $XXIX_3$	1a,1b,1c
29	—	—	$XXIX_1$, $XXIX_2$	1a,1b,1d
29	—	—	—	1a,1d,1e
29	—	—	—	(1a),1f
30	—	—	—	3

— No designation of antigens.

Table 10. Diagnostic single factor O serums

Antigenic factor	Serum	Absorbing strains
1a . . .	1a,1b,1c	2a,1b.
2a	2a,1b	1a,1b,1c.
3a	3a,3b,1c	1a,1b,1c+7,3b,1c.
4a	4a,4b	6,4b,5b.
5a	5a,5b,4b	6,4b,5b.
6	6,4b,5b	5a,5b,4b.
7	7,3b,1c	3a,3b,1c.
8a	8a,1c	1a,1b,1c.
8b	8a,8b	8a,8c.
8c	8a,8c	8a,8b.
9a	9a,9b	10,9b.
10	10,9b	9a,9b.
12b	12a,12b	12a,12c.
12c	12a,12c	12a,12b.
21a	21a,21b	30,21b.
28	28,1c	1a,1b,1c.
30	30,21b	21a,21b.

. . . Only a portion of the antigen represented.

positive. There was a reciprocal relationship between Bethesda-Ballerup type strain 11 and *Salmonella* XL and Arizona 10. The O type strains Arizona 10 and *Salmonella* XL have been found to be nearly identical (*14*) and, for all practical purposes, the reactions of Bethesda-Ballerup O type 11 in these two antiserums may be considered as one reaction. In tube agglutination tests the reciprocal cross-reactions did not exceed 10 percent of the homologous titers of the serums. There was a slight relationship between the Bethesda-Ballerup strain 12a,12c and the Arizona O type strains 1,2 and 1,3 which was evident in slide tests. The tube agglutination tests showed reciprocal cross-reactions to approximately 10 percent of their homologous titers. There was a minor nonreciprocal relationship between Bethesda-Ballerup O 14 and *Salmonella* XXXVIII, in which the Bethesda-Ballerup strain was agglutinated to one-tenth of the titer of the *Salmonella* antiserum. Bethesda-Ballerup O 20 type strains and *Salmonella* XVII and Arizona 12 type strains showed strong reciprocal agglutination in slide tests, yet tube agglutination tests revealed only a minor relationship. Bethesda-Ballerup O 21a,21b type strain was agglutinated only slightly in *Salmonella* serum

Table 11. Slide agglutination tests—Bethesda-Ballerup O type strains in *Salmonella* and Arizona O antiserums

O antigens	O antiserums												
Bethesda-Ballerup	*Salmonella*	IV,V,XII	VI,VII	VI,XIV,XXIV	VI,XIV,XXV	XVII	XVIII	VIII,XX	XXX	XXXVIII	XL		
	Arizona	—	—	—	—	12	7	—	—	—	10	1,2	8
9a,9b		—	—	—	—	—	—	—	++	—	—	—	—
11		—	—	—	—	—	—	—	—	—	+	—	—
12a,12c		—	—	—	—	—	—	—	—	—	—	+	—
14		—	—	—	—	—	—	±	—	++	—	—	—
20		—	—	—	—	++	—	—	—	—	—	—	—
21a,21b		—	—	±	—	—	—	—	—	—	—	—	—
22		++	—	—	—	—	—	—	—	—	—	—	—
23		—	—	—	—	—	++	—	—	—	—	—	—
28,1c		—	++	—	++	—	—	++	—	—	—	—	—
32		—	—	—	—	—	—	—	—	—	—	—	+

— No agglutination.
± to ++ degree of agglutination.

Table 12. Slide agglutination tests—*Salmonella* and Arizona O type strains in Bethesda-Ballerup O antiserums

O antigens		Bethesda-Ballerup O antiserums													
Salmonella	Arizona	3a,3b,1c	9a,9b	11	12a,12b	12a,12c	19	20	21a,21b	22	23	24	26	31	32
I,II,XII	—	—	—	+	—	—	—	—	+	—	—	—	—	—	—
IV,V,XII	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—
I,IV,XXVII, XII	—	—	—	—	—	—	—	—	—	++	—	—	—	—	—
VI,VII	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—
VI,VIII	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—
IX,XII	—	—	—	—	—	—	±	—	—	—	—	—	—	—	—
III,X	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
VI,XIV,XXIV	—	—	—	+	—	—	—	—	++	—	—	—	—	++	—
(I),VI,XIV,XXV	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—
XVII	12	—	—	—	—	—	—	++	—	—	—	—	—	—	—
XVIII	7	—	—	—	—	—	—	—	—	—	+	—	—	—	—
XXI	—	—	—	—	—	—	—	—	—	—	—	—	++	—	—
XXVIII	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—
XXX	—	—	++	—	—	—	—	—	—	—	—	—	—	—	—
XL	10	—	—	++	—	—	—	—	—	—	—	—	—	—	—
XLI	13	++	—	—	—	—	—	—	—	—	—	—	—	—	—
	1, 2	—	—	—	±	±	—	—	—	—	—	—	—	—	—
	1, 3	—	—	—	+	—	—	—	—	—	—	—	—	—	—
	8	—	—	—	—	—	—	—	—	—	—	—	—	—	±
	11	—	—	—	—	—	—	—	—	—	—	+	—	—	—

— No agglutination.
± to ++ degree of agglutination.

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VI,XIV,XXIV, yet the *Salmonella* type strain was agglutinated strongly in Bethesda-Ballerup O 21a,21b antiserum on the slide. This *Salmonella* O type strain also was agglutinated by Bethesda-Ballerup O 11 and O 31 antiserums although the reverse reactions did not occur.

The relationship which existed between Bethesda-Ballerup O 22 type strain and *Salmonella* group B strains was a strong reciprocal one in both the slide and the tube tests. This relationship is one of the most important ones which exists between the two groups, due to the frequent use of *Salmonella* grouping serums in diagnostic tests and to the known prevalence of *Salmonella* group B organisms. Bethesda-Ballerup O 23 shared a minor antigenic fraction with *Salmonella* O XVIII and Arizona O 7 which was evidenced by strong reciprocal slide reactions. Bethesda-Ballerup O 28,1c type strain was agglutinated strongly on the slide by three *Salmonella* antiserums, VI, VII; VIII, XX; and I, VI, XIV, XXV, yet the reverse reactions did not occur. There was a reciprocal relationship between the Bethesda-Ballerup O 32 type strain and that of Arizona O 8. In addition, the following nonreciprocal reactions of *Salmonella* and Arizona O type strains in Bethesda-Ballerup O antiserums were noted: I, II, XII in 11 and 21a,21b antiserums; IX, XII in 19 antiserum; III, X in 3a,3b,1c antiserum; XXI in 26 antiserum; XLI and Arizona 13 in 3a,3b,1c antiserum; and Arizona 11 in 25 antiserum.

Table 13 summarizes the positive results of tube agglutination reactions of Bethesda-Ballerup O type strains in *E. coli* O antiserums. Reciprocal titrations of *E. coli* antigens in Bethesda-Ballerup O antiserums were performed when indicated. As emphasized before, in all probability, the relationships observed were a reflection of only a portion of those which existed between these two groups of enteric bacteria. It is reasonable to assume that there are one-sided relationships as yet undetermined, but which would be revealed by the titration of all *E. coli* O group and O type antigens in all of the Bethesda-Ballerup O type antiserums.

Masking Antigens

The presence or prevalence of masking antigens, i. e., those heat labile somatic antigens which inhibit O agglutination, was not a point of intensive investigation in the present study. O inagglutinability of alcohol-treated antigens in antiserums prepared with boiled broth cultures occurred rarely in these organisms. However, the presence of several different masking antigens in Bethesda-Ballerup strains was recognized. The Vi antigen of Felix and Pitt long has been known to occur among enteric bacteria other than *S. typhi.* Its presence in the original Ballerup strain was described by Kauffmann and Moeller (*3*). Bruner and associates (*7*) demonstrated the presence of Vi antigen in strains of two of their Ballerup O groups (O groups 5 and 29 of this schema). These findings were confirmed in the present study. However, Vi antigen was not found in O groups other than 5 and 29. Two masking antigens other than Vi antigen were recognized in two Bethesda-Ballerup strains. One of these antigens is related to, but not identical with, the alpha antigen of Stamp and Stone (*15*). This alpha-like antigen was present in the type strain 3723-51 of O group 27. The O and H antigens of the Bethesda-Ballerup strain were unrelated to those of the alpha strain 1721 (Wakefield). The second strain which possessed a masking antigen, other than Vi antigen, was a member of O group 29. Its K antigen was not found to bear significant relationships to any known K antigen, including the recognized K antigens of *E. coli.*

Table 13. Relationships of Bethesda-Ballerup O antigens to those of *Escherichia coli* [1]

Bethesda-Ballerup	*E. coli*
1a,1b,1c	[1] *9*
5a,5b,4b	*122*
7,3b,1c	*99*
8a,8b	*93*
9a,9b	*7*
10,9b	71
12a,12c	*38*
15	*57*, 65
17	*101*
18	1, *15*, 100
21	*73*
26	*76*
28,1c	*9*, 44, 73
29	53, 62, 73
31	52

[1] When reciprocal relationships exist the *E. coli* O antigens are in italics.

Table 14. Relationships between type strains containing H antigen 2

H antiserums	H antigens	
	1,2	2,3
1,2:		
Unabsorbed	10, 000	500
Absorbed by 2,3	100	----------
2,3:		
Unabsorbed	2, 000	5, 000
Absorbed by 1,2	----------	500

H Antigens

H antigens of the Bethesda-Ballerup group. By agglutination and agglutinin-absorption tests, 88 flagellar antigenic fractions were distinguished. Seventy-five combinations of these 88 antigens occurred among cultures which belonged to the 32 O groups. For the H type strains and their antigenic formulas reference again is made to table 2. Twenty-nine flagellar types (39, 47, 48, 52, 56 through 73, 76, and 82 through 87) showed no significant cross-reactions with other flagellar antigens of the group.

Tables 14 through 26 present the cross-reactions and results of absorption experiments involving the groups of related flagellar type strains. For the most part, the relationships which existed among the flagellar type strains were more numerous and more complex than those which occurred among the somatic or O type strains. In some instances the relationships between H types were minor ones, as judged by cross-agglutination reactions, yet absorption experiments showed that the homologous titers were reduced markedly by absorption of the antiserums by heterologous strains. An example of this is shown in table 14 which presents the results of agglutination and agglutinin absorption tests with flagellar types 1,2 and 2,3. H type 1,2 antiserum with a homologous titer of 1:10,000, agglutinated H type 2,3 only to 1:500, yet absorption of 1,2 antiserum by the H type 2,3 strain reduced the homologous titer of that antiserum to 1:100. The relationships between these two flagellar types were far stronger than was evident from the cross agglutination demonstrable in the unabsorbed antiserums of the two types. It is of interest as well as of practical importance to note that, in the examination of additional strains of these two H types, cultures were found which agglutinated to a high degree in both 1,2 and 2,3 antiserums. Absorption of each of the antiserums by such cultures showed them to be identical with one or the other of the two types. It can be reasoned from these results that, in some strains, the various flagellar components either are more sensitive to agglutination or are present in larger proportions than in other cultures.

The relationships that occurred between H types 4,5 and 5,6 are shown in table 15. Table 16 presents the relationships which existed between the strains of flagellar types 7, (8),10; 8; 8,9; 8,10,11; and 8,12. The specific fraction 7 was prepared by the absorption of antiserum 7,(8),10 by the type strains 8,9 and 8,10,11. Factor 8 may be demonstrated in a given strain by agglutination in the unabsorbed antiserum of type 8. The single factor 9 was prepared by the absorption of 8,9 antiserum by the 7,(8),10 type strain or by the 8 type strain. Factor 8 probably could be divided into two fractions since the 8 fractions contained by 8,9 and 8,10,11 were not identical. This was apparent in the absorption experiment in which 8,9 antiserum absorbed by 8,10,11 antigen still left agglutinins for 7,(8),10, and 8 type strains. The specific factor 11 may be prepared by the absorption of 8,10,11 antiserum by 7,(8),10 plus 8,9 type strains. Factor 12 was prepared by the absorption of 8,12 antiserum by the 8,10,11 type strain.

Table 17 shows the relationships between six closely integrated H type strains. Three of these H types exhibited strong reciprocal rela-

Table 15. Relationships between strains containing H antigen 5

H antiserums	H antigens	
	4,5	5,6
4,5:		
Unabsorbed	10, 000	100
Absorbed by 5,6	5, 000	----------
5,6:		
Unabsorbed	200	10, 000
Absorbed by 4,5	----------	5, 000

Table 16. Relationships among strains containing H antigen 8

H antiserums	H antigens				
	7,(8),10	8	8,9	8,10,11	8,12
7,(8),10:					
Unabsorbed	10, 000	500	1, 000	1, 000	----------
Absorbed by—					
8	10, 000	----------	200	----------	----------
8,9	5, 000	----------	----------	1, 000	----------
8,10,11	5, 000	----------	100	----------	----------
8,9+8,10,11	5, 000	----------	----------	----------	----------
8:					
Unabsorbed	200	5, 000	5, 000	10, 000	200
Absorbed by—					
7,(8),10	----------	1, 000	1, 000	1, 000	----------
8,10,11	----------	200	100	----------	----------
8,9	----------	----------	----------	----------	----------
8,9:					
Unabsorbed	1, 000	2, 000	5, 000	1, 000	200
Absorbed by—					
8	200	----------	2, 000	----------	----------
7,(8),10	----------	1, 000	2, 000	200	----------
8,10,11	200	500	1, 000	----------	----------
7,(8),10 + 8	----------	----------	1, 000	----------	----------
8,10,11:					
Unabsorbed	2, 000	5, 000	2, 000	10, 000	500
Absorbed by—					
8,9	200	----------	----------	2, 000	----------
7,(8),10+8,9	----------	----------	----------	2, 000	----------
8,12	2, 000	500	500	5, 000	----------
8,12:					
Unabsorbed	100	500	500	5, 000	10, 000
Absorbed by 8,10,11	----------	----------	----------	----------	1, 000

tionships (types (9),13,14; (9),13,15; and 14,-15,16). The first two types listed did not possess H antigenic fractions which were not present in other H types. By the absorption of antiserum (9),13,14 with the (9),13,15 type strain, the fraction specific for factor 14 was prepared. Serum for factor 15 was prepared by the absorption of (9),13,15 antiserum by (9),13,14 plus (13), 17 type strains; factor 16 by the absorption of 14,15,16 antiserum by both of the type strains (9),13,14 and (9),13,15. The latter two absorbing strains also were used in the preparation of the single factor 17 from (13),17 antiserum. They were necessary along with type strain 19,20 for the preparation of factor 18 in the absorption of antiserum (13),18,19. The relationship between the (13),-18,19 and the 19,20 type strains and the previously discussed types within this group of related strains illustrated a type of antigenic behavior which was not uncommon among these paracolon organisms. Type strain (13),-18,19 antigen was agglutinated in only one of the antiserums (19,20) prepared against the other five organisms of this H group and, in that one instance, to only one-fiftieth of the homologous titer. Yet an antiserum prepared against the strain of H type (13),18,19 agglutinated the type strains (9),13,14 and (9),13,15 to a high titer. It agglutinated the 19,20 type to one-half of its homologous titer. The single factor 20 was prepared by absorbing 19,20 antiserum by the type strain (13),18,19.

The relationships which existed among the type strains containing H antigen 21 are presented in table 18. As was the case in the preceding group of related strains, nonreciprocal and minor relationships existed in this group of five strains. As is evident from the data given in table 18, factor 21 varied quantitatively or actually differed qualitatively in cultures of this H group. It was necessary to absorb 21,22 antiserum by the 21,23 H type strain in order to prepare the specific factor 22. Absorption of

21,22 antiserum by 21,24 H type strain failed to remove all agglutinins for the 21,23 H type strain. Single factor 23 serum was prepared by the absorption of 21,23 antiserum by the H type strain (21),25,26, while single factor 24 serum was prepared by the absorption of 21,24 serum by the H type strain 21,22.

Specific antiserums for the H factors 26 and 27 were obtained by the absorption of (21),25,26 and (21),25,27 antiserums by the H type strains (21),25,27 and (21),25,26 respectively.

The relationships between the two H type strains (9),29,30 and (9),29,31 were not reciprocal ones. Table 19 presents the cross agglutination reactions of these strains and the preparation of the specific H factor serums 30 and 31. It is of interest to note that although (9),29,30 type strain was not agglutinated by (9),29,31 antiserum the homologous titer of this serum was reduced by absorption with the (9),29,30 type strain. The relationships between the H type strains 32,33 and 32,34 and the preparation of the specific factor serums 33 and 34 are shown in table 20.

Table 21 presents the relationships found among the strains containing H antigen 35 and shows the agglutinin absorptions necessary for the preparation of the single factor H serums, 36,37 and 38. Single factor 36 was prepared by the absorption of 35,36 antiserum by the 35,37 H type strain. The possession of only a portion of the 36 factor by the H type strain 35,(36),38 was evident from a comparison of the agglutination titers obtained when H type strains 35,36 and 35,(36),38 were titrated in single factor 36 serum. The titer of 35,(36),38

Table 17. Relationships among strains containing H antigens 13, 15, and 19

H antiserums	H antigens					
	(9),13,14	(9),13,15	14,15,16	(13),17	(13),18,19	19,20
(9),13,14:						
Unabsorbed	20, 000	5, 000	5, 000	----------	----------	----------
Absorbed by—						
14,15,16	5, 000	2, 000	----------	----------	----------	----------
(9),13,15	200	----------	200	----------	----------	----------
14,15,16+(9),13,15	----------	----------	----------	----------	----------	----------
(9),13,15:						
Unabsorbed	10, 000	20, 000	5, 000	500	----------	----------
Absorbed by—						
(9),13,14	----------	2, 000	2, 000	500	----------	----------
14,15,16	10, 000	20, 000	----------	500	----------	----------
(13),17	10, 000	5, 000	5, 000	----------	----------	----------
(9),13,14+14,15,16+(13),17	----------	----------	----------	----------	----------	----------
14,15,16:						
Unabsorbed	10, 000	10, 000	20, 000	----------	----------	----------
Absorbed by—						
(9),13,15	500	----------	2, 000	----------	----------	----------
(9),13,14+(9),13,15	----------	----------	2, 000	----------	----------	----------
(13),17:						
Unabsorbed	2, 000	2, 000	----------	20, 000	----------	----------
Absorbed by—						
(9),13,15	500	----------	----------	10, 000	----------	----------
(9),13,14+(9),13,15	----------	----------	----------	10, 000	----------	----------
(13),18,19:						
Unabsorbed	5, 000	2, 000	----------	----------	40, 000	20, 000
Absorbed by—						
(9),13,14	----------	1, 000	----------	----------	20, 000	20, 000
(9),13,15	2, 000	----------	----------	----------	20, 000	20, 000
19,20	2, 000	500	----------	----------	20, 000	----------
(9),13,14+(9),13,15+19,20	----------	----------	----------	----------	20, 000	----------
19,20:						
Unabsorbed	----------	----------	----------	----------	200	10, 000
Absorbed by (13),18,19	----------	----------	----------	----------	----------	5, 000

Table 18. Relationships among strains containing H antigen 21

H antiserums	H antigens				
	21,22	21,23	21,24	(21),25,26	(21),25,27
21,22:					
Unabsorbed	5, 000	2, 000	2, 000	----------	----------
Absorbed by—					
21,23	500	----------	----------	----------	----------
21,24	2, 000	1, 000	----------	----------	----------
21,23:					
Unabsorbed	100	10, 000	100	500	100
Absorbed by (21),25,26	----------	5, 000	----------	----------	----------
21,24:					
Unabsorbed	2, 000	100	10, 000	----------	----------
Absorbed by 21,22	----------	----------	5, 000	----------	----------
(21),25,26:					
Unabsorbed	1, 000	100	1, 000	20, 000	5, 000
Absorbed by (21),25,27	----------	----------	----------	2, 000	----------
(21),25,27:					
Unabsorbed	2, 000	200	1, 000	20, 000	10, 000
Absorbed by—					
21,22	----------	----------	----------	1, 000	5, 000
(21),25,26	----------	----------	----------	----------	5, 000

H type strain in 36 antiserum was one-half that obtained with the 35,36 H type strain. In addition, the H type strain 35,(36),38 failed to remove all homologous agglutinins from 35,36 antiserum. Single factor 37 was prepared by the absorption of 35,37 antiserum by either of the 35,36 or 35,(36),38 H type strains, since both strains contained the full complement of factor 35. Single factor 38 was prepared by absorbing 35,(36),38 antiserum by the H type strain 35,36.

The relationships among the three H type strains (40,41; 40,42; 40,41,43) containing H antigen 40, given in table 22, were most apparent in the agglutination of the 40,42 and 40,41,-43 type strains by antiserum 40,41. The other two antiserums did not agglutinate heterologous strains to any extent and the 40,42 type strain failed to agglutinate in the 40,41,43 antiserum at a dilution of 1:100. The reactions between these three H type strains and their respective antiserums was another illustration of the partial and nonreciprocal relationships which existed between members of the Bethesda-Ballerup group. The detection of the H antigenic fraction 41 in Bethesda-Ballerup cultures was accomplished with the use of a single factor 41 antiserum prepared by the absorption of 40,41 antiserum by the 40,42 H type strain. This single factor serum agglutinated both 40,41 and 40,41,43 H type strains. Single factor 42 serum was prepared by the absorption of 40,42 antiserum by the 40,41,43 H type strain. The specific serum for H factor 43 was prepared by the absorption of 40,41,43 antiserum by the H type strain 40,41.

Table 23 shows the relationships between the two strains which contained H antigen 44 and gives the absorptions necessary for the preparation of single factor serums for H antigens 45 and 46. The relationship between the two H type strains containing the antigen 49 is presented in table 24. This relationship was actually a one-sided one; the H type strain 49,50

Table 19. Relationships among strains containing H antigens 9 and 29

H antiserums	H antigens	
	(9),29,30	(9),29,31
(9),29,30:		
Unabsorbed	10, 000	1, 000
Absorbed by (9),29,31	5, 000	----------
(9),29,31:		
Unabsorbed	----------	20, 000
Absorbed by (9),29,30	----------	10, 000

Table 20. Relationships between strains containing H antigen 32

H antiserums	H antigens	
	32,33	32,34
32,33:		
Unabsorbed	20, 000	1, 000
Absorbed by 32,24	10, 000	----------
32,34:		
Unabsorbed	2, 000	20, 000
Absorbed by 32,33	----------	10, 000

Table 21. Relationships among strains containing H antigen 35

H antiserums	H antigens		
	35,36	35,37	35,(36),38
35,36:			
Unabsorbed	5, 000	200	1, 000
Absorbed by—			
35,37	1, 000	--------	500
35,(36),38	200	--------	----------
35,37:			
Unabsorbed	1, 000	5, 000	1, 000
Absorbed by—			
35,36	--------	1, 000	----------
35,(36),38	--------	1, 000	----------
35,(36),38:			
Unabsorbed	5, 000	2, 000	10, 000
Absorbed by—			
35,36	--------	--------	2, 000
35,37	1, 000	--------	5, 000

was agglutinated by 49,51 antiserum at a dilution of only 1:100, whereas the H type strain 49,51 was agglutinated at a dilution of 1:1,000 by antiserum 49,50. The absorption of the two antiserums by the heterologous strains did not reduce demonstrably their homologous titers.

Table 25 gives the cross-reactions which occurred between the two H type strains 53,54 and 53,55 and their respective antiserums and shows the absorptions necessary for the preparation of the factors 54 and 55.

The relationship among the six H type strains 77; 77,78; 77,79; 77,80; 77,81; and 77,88 and the preparation of single factor serums necessary for type determination are presented in table 26. The relationships among these strains were quite complex and, for the sake of simplification, not all of the fractions were represented by antigenic symbols. Although the factor 77 apparently was present to some degree in all six of these strains, it often was manifested only by nonreciprocal reactions. It also was evident that the fraction 77 could be expressed as being composed of two distinct components. Antiserum 77 agglutinated the H type strains 77,78; 77,79; and 77,80 but did not agglutinate the strains 77,81 or 77,88 at a dilution of 1:100. This antiserum was exhausted of all demonstrable H agglutinins by absorption with either H type strains 77,78 or 77,79 and therefore can be said to have no specific fraction of its own. Antiserum 77,78 agglutinated the heterologous strains 77 and 77,79 to 20 percent of its titer, agglutinated 77,80 to only 1:100, and failed to agglutinate 77,81 or 77,88 H type strains. When antiserum 77,78 was absorbed by the H type strain 77, a fraction specific for the antigen 78 remained. It was apparent that the H type strain 77,79 did not contain the full complement

Table 22. Relationships among strains containing H antigen 40

H antiserums	H antigens		
	40,41	40,42	40,41,43
40,41:			
Unabsorbed	20, 000	2, 000	5, 000
Absorbed by—			
40,42	2, 000	--------	1, 000
40,41,43	100	--------	----------
40,42:			
Unabsorbed	100	5, 000	200
Absorbed by 40,41,43	--------	5, 000	----------
40,41,43:			
Unabsorbed	500	--------	5, 000
Absorbed by 40,41	--------	--------	1, 000

Table 23. Relationships between strains containing H antigen 44

H antiserums	H antigens	
	44,45	44,46
44,45:		
Unabsorbed	10, 000	5, 000
Absorbed by 44,46	5, 000	----------
44,46:		
Unabsorbed	2, 000	10, 000
Absorbed by 44,45	----------	5, 000

Table 24. Relationships between strains containing H antigen 49

H antiserums	H antigens	
	49,50	49,51
49,50:		
Unabsorbed	10, 000	1, 000
Absorbed by 49,51	10, 000	----------
49,51:		
Unabsorbed	100	10, 000
Absorbed by 49,50	----------	10, 000

Table 25. Relationships between strains containing H antigen 53

H antiserums	H antigen	
	53,54	53,55
53,54:		
Unabsorbed	20, 000	2, 000
Absorbed by 53,55	5, 000	----------
53,55:		
Unabsorbed	500	10, 000
Absorbed by 53,54	----------	5, 000

of the 77 fraction possessed by the 77,78 H type since absorption of 77,78 antiserum by 77,79 H type strain still left agglutinins for the H type strains 77 and 77,80. The possession of a common fraction by these six strains was most apparent by their reactions in antiserum 77,79. All six strains were agglutinated to varying degrees in that serum. The 77 and 77,78 H type strains were agglutinated to the homologous titer of 1:20,000 of the antiserum; strain 77,80 was agglutinated to 20 percent of its titer, while strains 77,81 and 77,88, respectively, were agglutinated to 5 and 10 percent of the homologous titer. Absorption of 77,79 antiserum by the 77 H type strain left a small specific fraction for the factor 79. Absorption of antiserum 77,79 by either 77,78 or 77,88 type strains failed to remove all nonspecific agglutinins from this antiserum. Antiserums 77,80 and 77,81 agglutinated only their homologous strains and were used without absorption as specific serums for the factors 80 and 81, respectively. A relatively strong non-

Table 26. Relationships among strains containing H antigen 77

H antiserums	H antigens					
	77	77,78	77,79	77,80	77,81	77,88
77:						
Unabsorbed	20, 000	10, 000	10, 000	2, 000	----------	----------
Absorbed by—						
77,78	----------	----------	----------	----------	----------	----------
77,79	----------	----------	----------	----------	----------	----------
77,78:						
Unabsorbed	5, 000	20, 000	5, 000	100	----------	----------
Absorbed by—						
77	----------	500	----------	----------	----------	----------
77,79	500	5, 000	----------	100	----------	----------
77,79:						
Unabsorbed	20, 000	20, 000	20, 000	5, 000	1, 000	2, 000
Absorbed by—						
77	----------	----------	200	----------	----------	----------
77,78	500	----------	2, 000	----------	----------	----------
77,88	200	100	500	200	----------	----------
77,80:						
Unabsorbed	----------	----------	----------	10, 000	----------	----------
Absorbed by 77,79	----------	----------	----------	10, 000	----------	----------
77,81:						
Unabsorbed	----------	----------	----------	----------	5, 000	----------
77,88:						
Unabsorbed	200	----------	200	200	----------	10, 000
Absorbed by 77,79	----------	----------	----------	----------	----------	10, 000

reciprocal relationship was apparent between strains 77,79 and 77,80 in which the 77,80 H type strain was agglutinated in 77,79 antiserum.

Antiserum 77,88 possessed a portion of the common factor 77 as shown by its agglutination of the H type strains 77; 77,79; and 77,80. Since the amount of the antigen 77 may vary among different strains of the 77,88 type, absorbed antiserum should be used to detect the specific fraction 88. For this single factor serum, antiserum 77,88 was absorbed by the 77,79 H type strain.

The antigenic formulas assigned to Bethesda-Ballerup H type strains in this and previous reports are presented in table 27.

The methods for the preparation of the single factor H antiserums are summarized in table 28. It will be noted that certain factors are omitted from the list since their occurrence in a given strain was evidenced only by the fact that the culture was agglutinated in two or more antiserums which contained agglutinins for the factor. It is not possible to prepare antiserums specific for such common factors.

Relationships of H antigens to Those of Other Enteric Bacteria. No relationships were found between the flagellar antigens of the Bethesda-

Table 27. Antigenic formulas assigned Bethesda-Ballerup H type strains in previous reports

Suggested designation			
Bethesda-Ballerup West, Edwards 1954	Bethesda Moran, Bruner 1949	Bethesda Edwards, West, Bruner 1948	Ballerup Bruner, Edwards, Hopson 1949
1,2	1	1	—
4,5	2	2	—
5,6	3	3	—
7,(8),10	4	4	—
8	5	5	—
8,9	5,6	5,6	—
8,10,11	—	—	14
(9),13,14	6,(7),(8)	6	—
(9),13,15	6,7,8	—	—
14,15,16	7,9	—	—
(13),17	8,10	—	—
19,20	16	—	—
21,22	12	7	—
21,24	18	—	—
(21),25,26	13	—	—
(9),29,30	20	—	—
(9),29,31	11	—	—
32,33	15	—	—
32, 34	14	—	—
35,37	19	—	—
39	17	—	—
40,42	—	—	13
73	—	—	1
74	—	—	2
75	—	—	3
74,75	—	—	2,3
76	—	—	(3),4
77	—	—	5,7
77,79	—	—	5,6
77,80	—	—	(5),8
77,81	—	—	(5),9
82	—	—	10
83	—	—	11
84	—	—	12
85	—	—	15
86	—	—	16
87	—	—	17

Table 28. Diagnostic single factor H serums

Antigenic factor	Serum	Absorbing strains
1	1,2	2,3.
3	2,3	1,2.
4	4,5	5,6.
6	5,6	4,5.
7	7,(8),10	8,9+8,10,11.
8	8	Unabsorbed.
9	8,9	8+7,(8),10.
10 . . .	8,10,11	8.
11	8,10,11	8,9+7,(8),10.
12	8,12	8,10,11.
14	(9),13,14	(9),13,15.
15	(9),13,15	(9),13,14.
16	14,15,16	(9),13,14+(9),13,15.
17	(13),17	(9),13,14+(9),13,15.
18	(13),18,19	(9),13,14+(9),13,15+19,20.
19 . . .	(13),18,19	Unabsorbed.
20	19,20	(13),18,19.
21 . . .	21,22	Unabsorbed.
22	21,22	21,23.
23	21,23	(21),25,26.
24	21,24	21,22.
25 . . .	(21),25,27	21,22.
26	(21),25,26	(21),25,27.
27	(21),25,27	(21),25,26.
28	(23),28	21,23.
29 . . .	(9),29,30	Unabsorbed.
30	(9),29,30	(9),29,31.
31	(9),29,31	(9),29,30.
33	32,33	32,34.
34	32,34	32,33.
36	35,36	35,37.
37	35,37	35,36.
38	35,(36),38	35,36.
41	40,41	40,42.
42	40,42	40,41,43.
43	40,41,43	40,41.
44 . . .	44,45	Unabsorbed.
45	44,45	44,46.
46	44,46	44,45.
49 . . .	49,50	Unabsorbed.
50	49,50	49,51.
51	49,51	49,50.
53 . . .	53,54	Unabsorbed.
54	53,54	53,55.
55	53,55	53,54.
77 . . .	77,79	Unabsorbed.
78	77,78	77.
79	77,79	77.
80	77,80	77,79.
81 . . .	77,81	Unabsorbed.
88	77,88	77,79.

. . . Only a portion of the antigen represented.

Ballerup type strains and those of the Arizona or *Salmonella* groups. However, three related Bethesda-Ballerup type strains 40,41; 40,42; and 40,41,43 were agglutinated to 10 percent of the titer of *E. coli* H 13 antiserum.

Antigenic Classification

After the O and H antigens of the 506 strains were determined, an antigenic schema was established. In setting up the schema, the organisms were divided into groups on the basis of their O antigens. The O groups were divided further into antigenic types according to the H antigens of the cultures. In this way, 167 antigenic types were established. The schema is presented in table 29. Also included in the table are the number of strains of each type and the sources of the cultures of the individual types insofar as the latter could be ascertained.

Table 29. Antigenic schema and sources of serologic types

Types	Sources	Number of cultures
1a,1b,1c: 1,2	Stools: Cases, 4; asymptomatic, 3; unknown, 5	12
1a,1b,1c: 14,15,16	Stools: Asymptomatic, 1; unknown, 3; no history, 1	5
1a,1b,1c: (21),25,26	Stool: Unknown	1
1a,1b,1c: (25),21,27	No history	1
1a,1b,1c: 39	Stool: Unknown	1
1a,1b,1c: (9) . . .	Stool: Unknown	1
2a,1b: 1,2	Stool: Unknown	2
2a,1b: 5,6	Stools: Case, 1; asymptomatic, 4; unknown, 3; no history, 2	10
2a,1b: 7,(8),10	Stool: Case	1
2a,1b: 8,10,11	Stools: Cases	2
2a,1b: 14,15,16	No history	1
2a,1b: (13),17	Stools: Case 1; no history, 1	2
2a,1b: 21,22	Stools: Cases, 3; asymptomatic, 7; unknown, 4; peritonitis, 1; monkey, 1; no history, 2.	18
2a,1b: (23),28	Stool: Unknown	1
2a,1b: 32,34	Stools: Case, 1; unknown, 3	4
2a,1b: 35,37	Blood	1
2a,1b: 39	Stool: Unknown	1
3a,3b,1c: 4,5	Stools: Case, 1; asymptomatic, 2; unknown, 5; fowl, 1	9
3a,3b,1c: 5,6	Stools: Cases, 3; asymptomatic, 4; unknown, 4; no history, 3	14
3a,3b,1c: 7,(8),10	Stools: Cases, 4; unknown, 2; no history, 1	7
3a,3b,1c: 8	Stools: Cases, 2; unknown, 2; no history, 1	5
3a,3b,1c: 8,9	Stools: Cases, 3; asymptomatic, 1; unknown, 13; water, 1; no history, 3	21
3a,3b,1c: (9),13,14	Stools: Cases	2
3a,3b,1c: 14,15,16	Stools: Unknown, 1; no history, 1	2
3a,3b,1c: (13),17	Stools: Cases, 10; asymptomatic, 8; unknown, 6; fowl, 2; no history, 8	34
3a,3b,1c: 21,22	Stools: Case, 1; asymptomatic, 1; unknown, 2; no history, 2	6
3a,3b,1c: 21,23	Unknown	1
3a,3b,1c: 21,24	Stool: Case	1
3a,3b,1c: (21),25,27	Stools: Case, 1; no history, 1	2
3a,3b,1c: (9),29,30	Stool: Case	1
3a,3b,1c: (9),29,31	Stool: Asymptomatic	1
3a,3b,1c: 32,33	No history	2
3a,3b,1c: 32,34	No history	1
3a,3b,1c: 39	Stools: Cases, 5; asymptomatic, 3; unknown, 7; urine, 2; rat, 2; calf, 1; no history, 3.	23
3a,3b,1c: 47	Stool: Asymptomatic	1
3a,3b,1c: (4),(33) . . .	Fowl	2
3a,3b,1c: —	Mouse	1
4a,4b: 4,5	Stool: Case	1
4a,4b: 5,6	Stools: Cases, 3; asymptomatic, 5; unknown, 6; no history, 4	18
4a,4b: 7,(8),10	Stools: Cases, 2; asymptomatic, 2; unknown, 1	5
4a,4b: (9),13,14	Fowl	1
4a,4b: (13),17	Stools: Cases, 5; asymptomatic, 2; unknown, 1; fowl, 1	9
4a,4b: (13),18,19	Stools: Asymptomatic, 3; unknown, 2; no history, 2	7
4a,4b: 19,20	Stools: Cases, 2; asymptomatic, 1; unknown, 1; urine, 2; no history, 2	8
4a,4b: (9),29,30	Stool: Asymptomatic	1
4a,4b: (9),29,31	Stools: Cases, 7; asymptomatic, 3; unknown, 4; no history, 6	20

Table 29. Antigenic schema and sources of serologic types—Continued

Types	Sources	Number of cultures
4a,4b: 32,34	Stool: Unknown	1
4a,4b: 44,45	Stools: Case, 1; asymptomatic, 3; unknown, 1; monkey, 1; no history, 1	7
4a,4b: 44,46	Stool: Unknown	1
4a,4b: (31),(45) . . .	Stools: Asymptomatic	2
5a,5b,4b: 53,54	Stools: Case, 1; asymptomatic, 1; unknown, 2; hogs, 2; no history, 1	7
5a,5b,4b: 63	Stool: Unknown	1
5a,5b,4b: 73	Stool: Case	1
5a,5b,4b: —	Stool: Case	1
6,4b,5b: 72	Stool: Unknown	1
6,4b,5b: (54) . . .	Stool: Asymptomatic	1
7,3b,1c: 4,5	Stool: Case	1
7,3b,1c: 7,(8),10	Stools: Unknown, 1; no history, 1	2
7,3b,1c: 8,9	Stools: Cases	2
7,3b,1c: (9),13,14	Stools: Case, 1; unknown, 1; no history, 2	4
7,3b,1c: (13),17	Stools: Asymptomatic, 1; unknown, 2; dog, 1; no history, 2	6
7,3b,1c: 21,22	Stool: Unknown	1
7,3b,1c: 21,23	Stool: Case	1
7,3b,1c: (21), 25, 27	Dog	1
7,3b,1c: 39	Stools: Cases, 3; asymptomatic, 1; unknown, 1; no history, 1	6
7,3b,1c: 68	Stools: Asymptomatic, 1; no history, 1	2
7,3b,1c: (4), (33) . . .	Stool: Unknown	1
8a,1c: 1,2	No history	1
8a,1c: 5,6	Stools: Cases	2
8a,1c: (9),13,14	Stools: Cases, 3; asymptomatic, 1; unknown, 3	7
8a,1c: (9),13,15	Stool: Unknown	1
8a,1c: 21,22	Stool: Unknown	1
8a,1c: (21),25,27	No history	1
8a,1c: (9),29,30	No history	1
8a,1c: 32,33	Stools: Unknown, 5; no history, 1	6
8a,1c: 39	No history	1
8a,1c: 53,55	Stool: Asymptomatic	1
8a,1c: 67	Stool: Unknown	1
8a,8b: 1,2	Stools: Unknown, 2; water, 2	4
8a,8b: 8,12	No history	1
8a,8b: (9),13,14	No history	2
8a,8b: (21),25,26	Stools: Unknown, 1; elephant, 1	2
8a,8b: (21),25,27	Stools: Case, 1; asymptomatic, 2; unknown, 2	5
8a,8b: 35,37	Stool: Case	1
8a,8c: 5,6	Stools: Case, 1; unknown, 1	2
8a,8c: (9),13,14	No history	2
8a,8c: (13),17	Stools: Case, 1; asymptomatic, 1	2
8a,8c: (21),25,27	Stools: Case, 1; fowl, 1; no history, 1	3
8a,8c: 32,33	Stools: Case, 1; asymptomatic, 1; unknown, 2; no history, 3	7
8a,8c: 35,37	Stool: Asymptomatic	1
9a,9b: 1,2	No history	1
9a,9b: 2,3	Dog, 1; no history, 2	3
9a,9b: 4,5	Fowl	1
9a,9b: 8,9	No history	1
9a,9b: (13),17	Stools: Cases, 3; fowl, 3; no history, 1	7
9a,9b: (21),25,26	Stools: Unknown, 1; urine, 2	3
9a,9b: 32,33	No history	1
9a,9b: 32,34	Stool: Case	1
9a,9b: 39	Stools: Unknown, 1; dog, 1; no history, 3	5
9a,9b: 48	Stool: Case	1
9a,9b: —	Dog	1
10,9b: (13),17	No history	1
10,9b: (9),29,30	No history	1
10,9b: —	Stool: Asymptomatic	1

Table 29. Antigenic schema and sources of serological types—Continued

Types	Sources	Number of cultures
11: (9),13,14	No history	1
11: 14,15,16	No history	1
11: 32,33	Stool: Asymptomatic	1
11: 35,37	Stools: Cases	3
11: 35,(36),38	No history	2
11: 77,78	Stool: Unknown	1
11: 83	No history	1
12a,12b: 5,6	Stool: Case	1
12a,12b: (13),17	No history	1
12a,12b: 35,36	Stools: Cases	2
12a,12b: 57	Stool: Unknown	1
12a,12c: 57	Stool: Unknown	1
12a,12c: 62	Fowl	1
13: 5,6	No history	1
13: 59	Stools: Unknown, 1; no history, 1	2
13: 65	Stool: Asymptomatic	1
13: 66	Stools: Unknown, 1; no history, 1	2
13: 69	Stools: Case, 1; unknown, 1	2
14: 40,41	No history	1
14: 61	Stools: Cases	3
15: (13),18,19	Stool: Case	1
15: 21,22	Stool: Case	1
15: 32,34	No history	2
16: 21,24	Stool: Case	1
16: 58	No history	1
17: 21,24	Stool: Unknown	1
17: 44,45	No history	1
17: 75	Stool: Asymptomatic	1
18: 56	No history	2
19: 14,15,16	No history	1
19: 87	No history	1
20: 40,41,43	Stool: Unknown	1
21a,21b: 60	Rat	1
22: 64	Stool: Case	1
23: 52	No history	1
24: 49,51	Stool: Unknown	1
25: 35,(36),38	Stool: Unknown	1
26: 49,50	No history	1
26: 59	Dog	1
27: 40,41	No history	1
28,1c: 70	No history	1
29: 8,10,11	Stool: Case	1
29: 40,42	No history	1
29: 73	Stools: Cases, 2; unknown, 1; no history, 1	4
29: 74	Stools: Cases, 3; asymptomatic, 2; no history, 3	8
29: 74,75	Hog, 1; sewage, 1; no history, 1	3
29: 75	Fowl	1
29: 77,78	Dog	1
29: 77,79	Stools: Case, 1; unknown, 3; fowl, 1; no history, 1	6
29: 77	Stools: Asymptomatic, 1; oysters, 1; no history, 1	3

Table 29. Antigenic schema and sources of serological types—Continued

Types	Sources	Number of cultures
29: 77,80	Stool: Unknown	1
29: 77,81	No history	1
29: 77,88	Stool: Case	1
29: 82	Stool: Asymptomatic	1
29: 83	Stool: Case	1
29: 84	Stool: Unknown	1
29: 85	Stool: Asymptomatic	1
29: 86	Stool: Unknown	1
29: 87	Stools: Cases	2
29: —	Stools: Unknown, 8; no history, 1	9
30,21b: 76	Stools: Unknown, 1; no history, 1	2
31: 71	Stool: Unknown	1
32: (23),28	Stool: Asymptomatic	1
Total		506

Cases: Cultures from stools of persons said to be affected with diarrhea, gastroenteritis, or enteric fever.
Asymptomatic: Cultures isolated from apparently normal food handlers and from other persons not known to be affected with enteric disease.
Unknown: Strains known to have been isolated from human feces, but on which no information was obtained concerning the clinical condition of the persons from whom the stools were derived.
No history: Cultures sent to the laboratory with no information whatever. In all probability, most of these cultures were isolated from the stools of food handlers and of persons affected with diarrhea.

. . . Only a portion of the antigens represented.

Examination of *Escherichia freundii* Cultures

The biochemical characteristics of indol negative cultures presently classified as *Escherichia freundii* closely resemble those of the Bethesda-Ballerup group. Aside from the fact that the great majority of the Bethesda-Ballerup cultures ferment lactose slowly, whereas typical *E. freundii* cultures utilize the sugar rapidly, no differences are apparent in the biochemical properties of the two groups. Since the *E. freundii* group has not been classified by serologic methods, the writers were requested by Dr. F. Kauffmann, Copenhagen, Denmark, and by Dr. G. D'Alessandro, Palermo, Italy, to examine a number of indol negative cultures of *E. freundii* in their possession, making use of the antiserums prepared from the Bethesda-Ballerup strains. An account of the biochemical and serologic examination of the cultures follows.

Biochemical Tests

The IMViC reactions of the 79 *E. freundii* cultures were tested and their ability to blacken lead acetate papers was examined. The strains also were tested for fermentation of glucose, sucrose, lactose, salicin, and adonitol. Their reactions on TSI medium also were determined. All strains tested failed to form indol and were Voges-Proskauer negative and methyl red positive. All utilized Simmons' citrate within 1 to 4 days. All except four strains blackened lead acetate papers. Three of the 75 H_2S positive strains showed only slight H_2S production after 24 hours' incubation, the remaining strains showed abundant H_2S production at the end of 24 hours. The reactions on TSI agar were variable. All strains produced acid and gas in the butt of the tube, many strains also produced

Table 30. *Escherichia freundii* strains and sources

O	H	Sources	Number of cultures
4a,4b	68	Stool, diarrhea	1
4a,4b	73	Stool, typhoid case	1
5a,5b,4b	53,54	Stools, asymptomatic	3
5a,5b,4b	*	No history	1
5a,5b,4b	—	Stool, diarrhea	1
6,4b,5b	72	Water	1
6,4b,5b	37 . . .	Stool, typhoid case	1
6,4b,5b	—	----do	1
10,9b	(13),17	----do	1
10,9b	—	Stool, diarrhea	1
12a,12b	(13),17	Stool, unknown (1), ice cream (2)	3
12a,12b	(9),29,31	Sputum	1
12a,12c	—	Water filter	1
12a . . .	—	Sputum	1
13	(13),17	No history	1
13	(9),29,30	Stool, diarrhea	1
13	61,65	Water	1
15	(21),25,27	Stool, asymptomatic	1
17	44,45	Feces, rabbit	1
19	37 . . .	Stool, typhoid carrier	1
19	40,41	Stool, asymptomatic	1
19	—	----do	1
21a,21b	(9),29,31	Stool, diarrhea	1
21a,21b	40,41	Stool, asymptomatic	1
21a,21b	44,45	----do	1
21a,21b	53,54	Stools, typhoid case (1), asymptomatic (1)	2
21a,21b	—	Stool, asymptomatic	1
21a,21b	*	Stool, diarrhea	1
21a,21b . . .	21,24	Stool, asymptomatic	1
22	*	Stools, diarrhea (1), unknown (1)	2
24	85	Sputum (2), milk (1)	3
24	—	Water valve (1), tap water (1)	2
26	—	Surface water (1), tap water (3), nasal swab (1)	5
29, Vi	73	Stool, unknown	1
30,21b	37 . . .	----do	1
30,21b . . .	35 . . .	No history	1
32	(23),28	Water	1
—	(13),17	Sputum	1
—	53,54	Stool, typhoid case	1
—	84	Water (1), sputum (1)	2
—	*	Stool, diarrhea	1
—	—	Stools, typhoid cases (2), asymptomatic (4), unknown (3), appendix (1), appendical abscess (1), nose (1), throat (2), sputum (1), otitis (1), water (6).	22
Total			77

* Insufficient motility.
— Antigens unrecognized.
. . . Only a portion of the antigen represented.

Figures in parentheses in column 3 indicate number of strains from that source.

an acid slant. Nineteen strains blackened lead acetate papers but showed little or no H_2S production in TSI agar. All strains fermented glucose with acid and gas production within 24 hours. Lactose was fermented within 24 hours by 68 strains, within 48 hours by 7 strains, and within 72 hours by 2 strains. One strain required 4 days and another 6 days for the fermentation of lactose. Sucrose was fermented within 1 day by 30 strains, within 3 days by 1 strain, within 8 days by 2 strains, within 9 days by 2 strains, and within 14 days by 1 strain. Sucrose was not fermented by 43 strains within a 30-day period. Salicin was fermented within 1 day by 6 strains, within 2 days by 5 strains, within 3 days by 15 strains, within 5 days by 3 strains, within 7 to 9 days by 14 strains, and within 10 to 15 days by 12 strains. Four strains required 16 days or longer for the fermentation of salicin, while 20 strains failed to ferment this substrate within a 30-day period. Adonitol was not fermented by any of the 79 strains within a 30-day period.

Serologic Reactions

Alcohol-treated antigens of all of the *E. freundii* strains were prepared and tested on the slide with Bethesda-Ballerup O antiserums. In addition, broth cultures of the 35 strains received from Dr. Kauffmann were prepared in duplicate. One set was steamed for 1 hour, while the other set was autoclaved for 2½ hours. These steamed and autoclaved antigens were tested by the tube agglutination method at a dilution of 1:100 in all Bethesda-Ballerup O antiserums. The antigens which gave a positive reaction at a 1:100 dilution were titrated in the indicated antiserums. In all instances the results obtained by the tube test paralleled the results of the slide agglutination tests. The tube tests with the heated antigens were done to exclude the possibility of inhibition of O agglutination by heat labile somatic antigens. Only slide agglutination tests with alcohol-treated antigens were done with the remaining strains. Suspensions of the living organisms were tested on the slide with Vi antiserum. After maximum motility of these strains was assured by passage of the cultures through tall tubes of semisolid agar, formalinized broth cultures were prepared and used as H antigens in tube agglutination tests. When indicated, the H antigens were tested in single factor antiserums for complete H type identification.

The results of the serologic study of the *E. freundii* strains are presented in table 30, in which are included the sources of the strains. Two of these strains were so rough that their O antigens could not be recognized. The motility of 4 of the remaining 77 strains was so poor that the lack of agglutination in H antiserums could not be considered as significant. The O and H antigens of 33 strains were recognized by the use of Bethesda-Ballerup antiserums, although in 4 instances only a portion of the H antigens was identified. Twenty-one strains were shown by the use of absorbed antiserums to possess the complete O and H components of Bethesda-Ballerup cultures. In 14 strains only the O antigens were recognized and in 4 strains only the H antigens were recognized. Neither the O nor the H antigens were recognized in 22 of the smooth, actively motile cultures. Seventeen of the typable cultures represented serologic types not previously recognized. Vi antigen was recognized in 1 strain which belonged to O group 29.

There seemed to be no correlation between rapidity of lactose fermentation and serologic identification of these strains by Bethesda-Ballerup antiserums. Two of the 10 slow lactose fermenters were not typable in Bethesda-Ballerup antiserums. Of the 33 strains which were typable by these antiserums, 20 were rapid lactose fermenters. Also it was observed that of the 4 strains which failed to form H_2S as indicated by lead acetate papers, 2 possessed O and H antigens of the Bethesda-Ballerup group.

Polyvalent Antiserums

Several problems arise in the production of a polyvalent antiserum of optimal value in the preliminary or presumptive identification of a group of enteric bacteria. Too often results obtained with a polyvalent antiserum are regarded as absolute and final. The greater the antigenic coverage given by a polyvalent antiserum, the greater must be the care in the interpretation of the results obtained with that antiserum. The greater the number of strains used in the preparation of a polyvalent antiserum, the greater is the possibility of introducing agglutinins for antigenic fractions which are found in members of several groups of bacteria. There are known somatic relationships between Bethesda-Ballerup strains and some members of the *Salmonella*, Arizona, and *E. coli* groups. On the whole, the relationships are minor ones, yet they are detectable in slide agglutination tests in which diagnostic dilutions of O antiserums are used. Naturally, they are evident also in polyvalent serum.

In addition to the relationships which are known to exist between individual strains of different groups of bacteria, there are minor relationships which become apparent in polyvalent antiserums prepared with mixtures of a number of strains. In all probability, the magnification of these minor relationships in a polyvalent antiserum is due to the large number of antigens employed and to the prolonged course of immunization and the large doses used. Furthermore, it must be realized that different animals vary markedly in the production of agglutinins for minor antigenic fractions. This was illustrated forcibly in the preparation of polyvalent O antiserum in which antiserum from one rabbit possessed a high agglutinin titer for one of the *Salmonella* type strains, whereas another rabbit which received the same course of injection with the same antigens did not possess demonstrable agglutinins against this *Salmonella* strain.

The potential advantage of an H polyvalent antiserum for the Bethesda-Ballerup group lies in the fact that there are no known relationships between the flagellar antigens of Bethesda-Ballerup strains and those of members of the *Salmonella* and Arizona groups. These three groups of enteric bacteria often cannot be distinguished by the usual routine biochemical tests. That they are related by the possession of common O antigenic fractions further serves to complicate diagnosis. Because of the greater specificity of the polyvalent H antiserum, it would seem to be the preferred tool in screening. However, the poor motility of some strains and the consequent inagglutinability in H antiserums limit its usefulness.

Polyvalent O and polyvalent H antiserums which contained agglutinins for all of the recognized O and H antigens respectively were prepared. In the preparation of Bethesda-Ballerup polyvalent O antiserums, heated antigens of 35 O type strains were used for immunization of rabbits. These antigens were divided into three pools, each of which was administered to a different lot of rabbits. In order to prepare an antiserum which would agglutinate all 35 O types effectively it was necessary to use large amounts of antigens and to give at least 6 or 7 injections over a period of 5 or 6 weeks. After the animals were bled the 3 lots of serum were pooled. In the preparation of the polyvalent H antiserum, formalinized broth cultures of actively motile H type strains were mixed and used for the immunization of rabbits. As was the case in the preparation of the polyvalent O antiserums, the antigens were divided into three pools and large volumes of the mixtures were used and were injected over a long period of time.

These O and H polyvalent antiserums were used in tests with representatives of Bethesda-Ballerup and *Salmonella* type strains. The polyvalent O antiserum was used first on the slide with alcohol-treated antigens of members of both groups of bacteria. The polyvalent H antiserum was tested first in tube agglutination tests with formalinized broth cultures of actively motile H type strains of the Bethesda-Ballerup and *Salmonella* groups. Both polyvalent O and polyvalent H antiserums were used in slide tests with living organisms

representative of all Bethesda-Ballerup and *Salmonella* type strains. Also used in these slide tests with living organisms were two polyvalent O-H antiserums prepared in other laboratories against a limited number of the common serotypes of Bethesda-Ballerup strains. In addition, two *Salmonella* polyvalent antiserums were used in these tests. One of them was an O-H polyvalent serum prepared against all of the recognized O and H antigens of the *Salmonella* group. The second *Salmonella* polyvalent serum was a commercial product, in the preparation of which only representatives of *Salmonella* O groups A to E inclusive were used. In slide tests with both the Bethesda-Ballerup polyvalent O and H antiserums prepared in this laboratory, the type strains of that group were agglutinated strongly. However, both antiserums strongly agglutinated a number of *Salmonella* strains. The two simplified Bethesda-Ballerup O-H polyvalent antiserums failed to agglutinate the great majority of the Bethesda-Ballerup type strains and agglutinated only *Salmonella* strains representative of O group H. The two *Salmonella* polyvalent antiserums were effective for the purposes for which they were designed. They were included in these tests to determine the extent to which they agglutinated Bethesda-Ballerup type strains. The simplified commercial polyvalent antiserum agglutinated Bethesda-Ballerup strains of three O groups. The complete *Salmonella* polyvalent serum agglutinated Bethesda-Ballerup strains of nine O groups, not including O group 29. A Ballerup strain of the latter O group was included with the *Salmonella* strains in the preparation of the *Salmonella* polyvalent antiserum in order to produce a high agglutinin titer for Vi antigen. Inclusion of this strain resulted in a high titer for Bethesda-Ballerup antigens O 29 and H 73. As a consequence, any paracolon strain which contained these antigens was agglutinated strongly in this *Salmonella* polyvalent antiserum.

Tube agglutination tests were done only with the Bethesda-Ballerup polyvalent H serum prepared in this laboratory. Initially, each H type strain was titrated in this antiserum and all strains were agglutinated to a high titer. From these results, an effective diagnostic dilution of this antiserum for Bethesda-Ballerup strains was determined. Formalinized broth cultures of actively motile *Salmonella* H type strains were tested in the paracolon H polyvalent antiserum at the diagnostic dilution. No H agglutination occurred with any of the *Salmonella* strains.

Experience in routine screening of enteric bacteria with the polyvalent antiserums described above has been limited since the serums were not prepared until after the antigenic constitution of the group was determined and a diagnostic schema was established. For several years, pooled O antiserums and pooled H antiserums have been used in the laboratory with excellent results. However, to obtain the wide antigenic coverage desired it was necessary to use several O pools and several H pools and the method was too cumbersome to be used in the diagnostic laboratory.

Insofar as limited experience with polyvalent serums permits conclusions to be drawn, the following remarks seem justified. The polyvalent H serum used in tube tests has given specific results, and cultures which were agglutinated by it reacted with individual typing serums. Unfortunately many Bethesda-Ballerup cultures, when isolated, possess poorly developed H antigens and it is necessary to pass such cultures through semisolid agar before flocculation in H serum is apparent. The polyvalent H serum used in slide tests with living organisms acts as a combined O and H serum since agglutinins for many of the O groups are present. Used in this way the serum actively agglutinated all but 1 of 75 stock cultures of the Bethesda-Ballerup group, the motility of which had not been enhanced. These cultures contained all the recognized O and H antigens of the group. Agglutination of *Salmonella* cultures in parallel tests was much less marked than with the polyvalent Bethesda-Ballerup O antiserum.

From these results, it seems evident that a satisfactory screening procedure would be to examine suspected cultures simultaneously by slide agglutination with *Salmonella* polyvalent serum and Bethesda-Ballerup polyvalent H antiserum. Cultures which react in *Salmonella* polyvalent serum should be subjected to tube tests with *Salmonella* polyvalent H serum if

this reagent is available. Cultures which give positive slide tests with Bethesda-Ballerup H antiserum should be subjected to tube tests with the same serum and should be examined by appropriate biochemical methods.

It is probable that with greater experience it will be possible to prepare Bethesda-Ballerup polyvalent serums from which agglutinins for certain O groups are omitted. Some of the O groups which possess somatic relationships to frequently occurring *Salmonella* O groups appeared infrequently in the present series of Bethesda-Ballerup cultures. Thus, it should be possible to omit representatives of certain O groups and to improve the specificity of the antiserum while sacrificing little in the percentage of Bethesda-Ballerup cultures which would be detected.

Discussion

The principal purposes of this study were to provide a method for more rapid and exact group identification of paracolon organisms often confused with members of the genus *Salmonella*, and to provide a method for type determination within the group so that incidence of the individual types in normal carriers and in cases of enteric disease could be determined. Until the latter goal is attained it will not be possible to assess the pathogenic propensities of the organisms.

Biochemically, the Bethesda-Ballerup paracolon bacteria were characterized as gram negative, motile, nonspore-forming rods which reduced nitrates to nitrites; were methyl red positive and Voges-Proskauer negative; and produced hydrogen sulfide but failed to form indol or to liquefy gelatin. They utilized Simmons' citrate, and a few strains gave a positive reaction on Christensen's urea agar after overnight incubation. They produced acid and gas from glucose, xylose, maltose, mannitol, rhamnose, arabinose, and trehalose but failed to utilize adonitol. These organisms varied in their ability to utilize sucrose, lactose, dulcitol, and salicin. A few strains failed to produce demonstrable gas from fermentable substances.

In the light of the results of this study, the rapidity of lactose fermentation as a criterion by which an organism should be placed among the *Escherichia* or *Escherichia*-like groups, or placed among the paracolons or slow lactose fermenters, seemed to be a false one. Among Bethesda-Ballerup strains, there were both slow and rapid lactose fermenters and two strains of the same serologic type were shown to vary in the rapidity of lactose fermentation. One strain fermented lactose in 1 day, while the second strain required 4 to 8 days to utilize the sugar. This point again is brought out by the results of the biochemical and serologic examination of the 79 cultures sent to the laboratory as *E. freundii*. The great majority of these strains fermented lactose within 24 hours. This factor in itself apparently had no bearing upon whether a culture could be typed serologically by Bethesda-Ballerup antiserums. The fact that either the O or H antigens of approximately 70 percent of the smooth, motile cultures of *E. freundii* examined could be identified demonstrated the close relationship of the Bethesda-Ballerup group to the indol negative cultures presently classified as *E. freundii*. It appeared that the Bethesda-Ballerup group actually should be considered as a part of the indol-negative portion of the *E. freundii* group, the relationship being much the same as that of the *Escherichia*-like paracolons to the *E. coli* group. Inasmuch as the *E. freundii* group in general has not been subjected to intensive serologic study and since the cultures which ferment lactose promptly pose no problem in differentiation, this question has not been pursued further.

It is of interest to note that 95 percent or more of the non-*Salmonella* cultures which were received for reference diagnosis and which possessed biochemical properties characteristic of Bethesda-Ballerup strains were assignable to the O groups established in this work. Both

somatic and flagellar antigens have been recognized in approximately 85 percent of such cultures. It should be emphasized that not all of the somatic antigens, nor by any means all of the flagellar antigens, of this group of bacteria have been characterized in the present work. The total of 506 cultures included in this study did not represent all of the hundreds of Bethesda-Ballerup cultures which have been examined in this laboratory. It represents, rather, only those of which the exact antigenic structure had been confirmed since the antigenic schema was established.

Many cultures were studied which were assigned to O groups and which possessed H antigens related to those of the type strains, but the cultures were studied insufficiently to determine their exact formulas. There were a few cultures which did not belong to any of the established O groups but which possessed recognizable H antigens. Some cultures which belonged to definite O types possessed H antigens related to, but not identical with, the H antigens of type cultures. A few strains of the latter category were included in the schema with an indication that the complete H antigen was not represented.

In the identification of Bethesda-Ballerup cultures, those strains which possess biochemical characteristics of the group should be screened in polyvalent H antiserum in slide agglutination tests in which living organisms are employed as antigens. Those cultures which fail to react should be boiled and retested in the same manner. Separate O and H antigens should be prepared with those strains which give a positive reaction either before or after heating. It has been found useful in this laboratory to use pooled antiserums as an aid in rapid serologic identification of these organisms. The O serums are pooled and used in the slide test with alcohol-treated organisms as antigens. When positive reactions occur, the antigens are tested in the indicated individual O antiserums. When necessary for exact O antigen determination, single factor serums are employed. Due to the large number of H antigens which occur among the cultures, as well as to the poor motility of many of the strains, it has been found useful to employ polyvalent H antiserum in the tube test as the first step in H antigen identification. If no H agglutination is obtained, the strain should be passed through a series of tubes of semisolid agar for maximum development of the flagellar antigens, and the strain again tested in polyvalent H antiserum. Those cultures which then give a positive reaction are tested first in pooled and then in individual H antiserums. When indicated, single factor antiserums are employed for exact H type identification. Since the H antigens are so much more complex than the O antigens, often it is necessary to employ several absorbed H antiserums in order to identify completely the H antigens of a given strain.

The question of phase variation among Bethesda-Ballerup strains has not been a point of investigation in the present work. That it does occur among members of this group has been brought out in the work of Edwards and co-workers (*8*) in the original study of the Bethesda group. Monteverde and Leiguarda (*16*) and Edwards (*17*) showed that changes could be induced in the flagellar antigens of the Ballerup strains and that segregation of antigens occurred naturally in some cultures. The natural occurrence of phase variation and segregation of antigens, together with the possibility of induced changes in H antigens, may explain in part the complexity of the H antigens and the occurrence of many nonreciprocal H relationships. Previously, it had been demonstrated by Edwards and associates (*8*) that Bethesda strains of the same O group, isolated from the same individual, may possess H antigens which are of two types. Yet, by induced variation it was possible to change each of the H antigens to the other type. Thus, it is evident that two apparently distinct serologic types had an immediate common origin. The converse of this phenomenon in which a culture with complex H antigens naturally gave rise to two stable loss variants, each of which possessed a distinct portion of the H antigen of the parent culture, also has been observed (*17*). Such a situation can but compound confusion.

No data included in this study would prove or disprove the role of these organisms as primary causative agents of disease. They have been isolated occasionally from patients with urinary infections, and, in six instances, groups of persons affected with gastroenteritis each has yielded a single serologic type.

Unfortunately, the information available as to the sources of the majority of the cultures and the case histories of the individuals from whom the strains were isolated is incomplete. The information available as to the role of these organisms in epidemics is inconclusive and it is known from previous works (*8*) and from data included here that the percentage of these organisms in asymptomatic individuals is high. Seeliger (*18*) also noted the frequent occurrence of the bacteria in apparently healthy persons.

It is realized that the average laboratory has neither the time nor the facilities to attempt a complete serologic typing of these paracolon organisms. However, it is hoped that by outlining the biochemical limits of the group, as presently known, and by having available in reference diagnostic centers the means of complete identification of these organisms, some contribution has been made toward laying the foundation for the assessment of their role in enteric disease.

Summary

The Bethesda and Ballerup groups of paracolon bacteria were combined because of their similarity in biochemical properties and because of numerous serologic relationships which existed between the two groups.

Biochemical and serologic studies were made of 506 cultures of the combined groups. The biochemical limits of the group were established and an antigenic schema has been devised. The cultures were divisible into 32 O groups and 35 O types. The H antigens of the bacteria were more diverse, and 88 H antigens which occurred in 75 distinct combinations were established. On the basis of these O and H antigens, 167 serologic types were recognized. Among cultures of this group received for diagnosis, 95 percent could be assigned to established O groups and both O and H antigens of 85 percent of such cultures were recognized.

Polyvalent serums were prepared by different methods and their efficacy as diagnostic tools was studied.

Marked relationships of the Bethesda-Ballerup group to indol-negative cultures, classified as *Escherichia freundii*, were noted. Either the O or H antigens of 70 percent of the latter cultures could be recognized by use of antiserums prepared from Bethesda-Ballerup strains.

From the data at hand, no conclusions could be drawn concerning the role of the organisms in the production of enteric disease. Their frequent occurrence in the intestinal tract of apparently normal persons and animals was noted.

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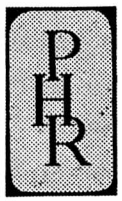

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